ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

VOLUME 85, ART. 3 PAGES 735–992

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CARE AND DISEASES OF THE RESEARCH MONKEY

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NEW YORK
PUBLISHED BY THE ACADEMY
May 12, 1960

The Sections and the Divisions hold meetings regularly, one evening each month, during the academic year, October to May, inclusive. All meetings are held at the building of The New York Academy of Sciences, 2 East Sixty-third Street, New York 21, New York.
Conferences are also held at irregular intervals at times announced by special programs.

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CARE AND DISEASES OF THE RESEARCH MONKEY*

Conference Chairman and Consulting Editor

ROBERT M. SAUER

CONTENTS

* This series of papers is the result of a conference on *Care and Diseases of the Research Monkey* held by The New York Academy of Sciences, November 19 and 20, 1959.

THE MONKEY: A COMPARISON OF THE NATURAL ENVIRONMENT WITH OBSERVATIONS IN CAPTIVITY

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Monkeys are essentially tropical mammals, and their distribution in the Old World, past and present, corresponds rather closely with that of early man. They also occur in the New World Tropics so that, collectively, they may be termed pantropical. Based on their usage in research, the most important families of Primates are the New World Cebidae and the Old World Cercopithecidae. Although all the members of these two families are readily recognized as "monkeys," even by laymen, the two divisions are quite different structurally, and scientists feel certain that they diverged at a very early date. It is generally felt that the New World Primates evolved from North American Eocene lemuroids or tarsioids, and any superficial resemblance to the Old World Cercopithecoids is a matter of parallelism.

Because of the structure of their nostrils, the New World monkeys have been designated platyrrhines. This is due to the broad transverse expansion of the anterior terminal portion of the septal cartilage, pushing the nostrils and their alar cartilages outward. By contradistinction, the Old World monkeys are termed catarrhines because in them this septal expansion is much reduced, and the nostrils are obliquely approximated. In dental formulae, the two families also differ—the Cebidae having 36 teeth, whereas the Cercopithecidae have only 32. The difference lies in the premolars, of which the Cebidae have three on each side of both jaws, whereas the Cercopithecidae have only two. The reduction in the number of teeth of the latter would seem to indicate their higher evolutionary position. Their dental formula is identical to that of man. Cheek pouches and bare ischial callosities are Old World monkey monopolies, whereas prehensile tails are exclusively New World. However, not all of the Cebidae possess them. They are confined to the howler, spider, woolly, and Cebus monkeys. There is increasing evidence that individual squirrel monkeys have a marked degree of prehensility in their caudal appendages. This genus (*Saimiri*) is considered by Simpson[1] to be related closely to *Cebus*.

New World primates show less adaptability than their Old World counterparts, for they are confined almost completely to forested areas, and are limited mainly to the Tropical Zone. They extend to approximately 21° north latitude in the Mexican states of Vera Cruz and San Luis Potosi. The northernmost-ranging nonhuman primate is the Mexican race of Geoffroy's spider monkey (*Ateles geoffroyi vellerosus*), which almost reaches the Tropic of Cancer. The Mexican mantled howler monkey (*Alouatta villosa mexicana*) reaches the southern part of Vera Cruz. In South America two Cebids cross the Tropic of Capricorn to enter the South Temperate Zone in Paraguay and extreme northern Argentina. They extend to approximately 27° south latitude in the vicinity of the junction of the rivers Parana and Paraguay. This is just a little beyond the 70° mean annual isotherm. Again, one is a howler—the black howler (*Alouatta caraya*), but spider monkeys are conspicuous by their absence in this

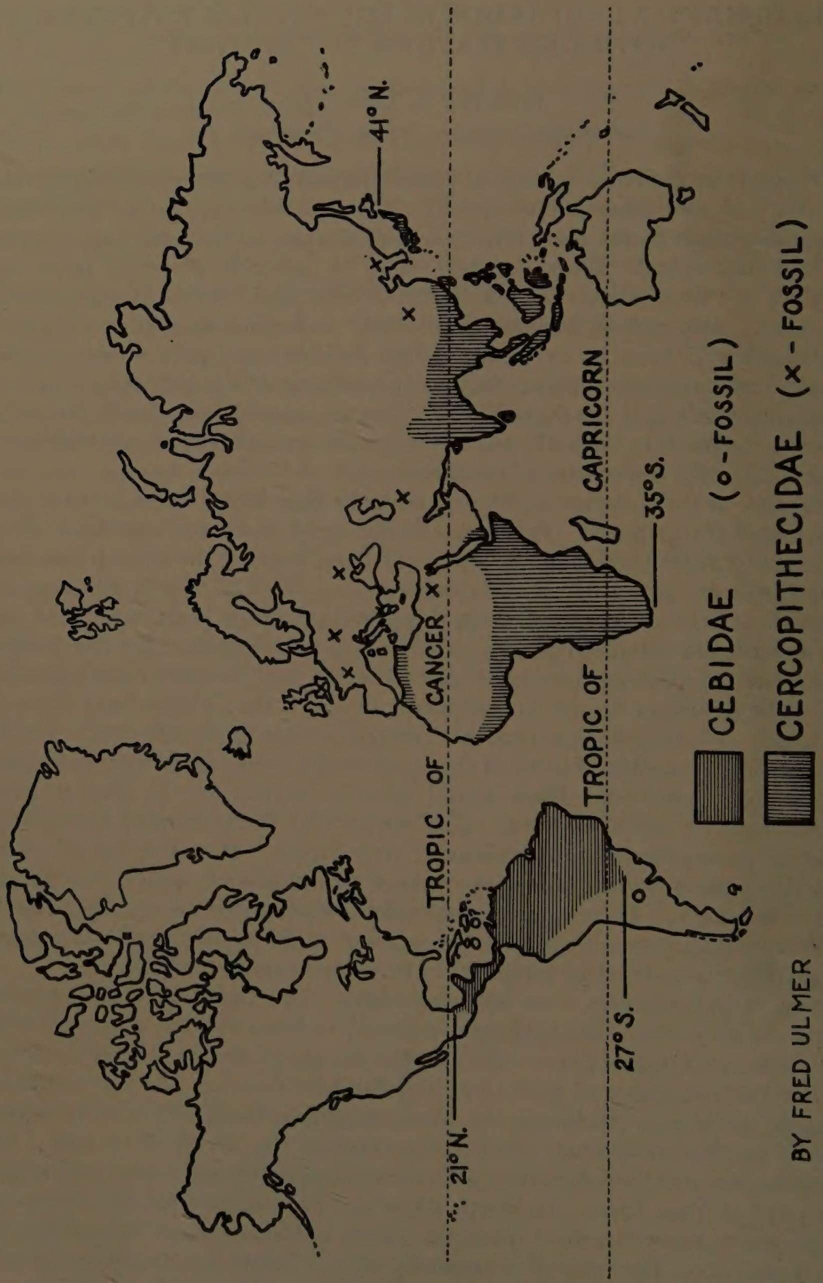

FIGURE 1. Map showing the distribution of the Cebidae in the New World and the distribution of the Cercopithecidae in the Old World.

southerly region. The other Cebid is Azara's capuchin (*Cebus azarae*)—a member of the tufted capuchin group. Altitudinally, the Cebids range up to about 6500 feet elevation. Spider and woolly monkeys have been recorded to about 6000 feet, and P. Hershkovitz states that the red howler is found to ap-

FIGURE 2. Geoffroy's Spider Monkey (*Ateles geoffroyi*) is an extremely arboreal example of the tree-loving Cebidae. It ranges farther north than any other New World monkey—approximately 21° north latitude in Mexico—but is confined to the Tropic Zone. Its greatly elongated extremities would result in rapid heat loss in a cool climate.

proximately 6500 feet in Colombia—higher than any other kind of monkey. Oddly enough, the seemingly very adaptable capuchin monkeys apparently reach their limits at about 4500 feet.

By comparison, the Old World monkeys show a far greater cold tolerance, ranging from 34° south latitude in Africa to 41° north latitude in Asia. They also exhibit a greater altitudinal range. In the South Temperate Zone of the Union of South Africa, two Cercopithecoids, the vervet monkey (*Cercopithecus*

aethiops pygerythrus) and the chacma baboon (*Papio comatus*) extend, or did until recently, to the southern limit of the continent. It is cool enough here for the inhabitants to recognize a winter season when cold rains occur, and snow is not uncommon at higher elevations. I might add that, in these settled districts, the chacma baboon is found only in the mountains, but it is an extremely rugged primate and it has been kept out of doors all winter in Philadelphia with no ill effects.

Farther north, African monkeys are widely distributed in the Tropic Zone, but they still show remarkable abilities to withstand extremes of temperature for, altitudinally, several species range into the Temperate Zone. The black and white *Colobus* monkey is especially noteworthy, for it reaches elevations of 9000 feet or more on Mt. Kenya and Mt. Kilimanjaro, both in East Africa. The Sahara Desert forms an effective barrier to monkeys, but one species survives along the Mediterranean littoral of Morocco and Algeria. This robust, thickly furred, tailless macaque, the so-called Barbary ape, is admirably suited to a cooler climate and ranges as far as 37° north latitude.

The most successful monkeys in the world today are, I feel, the members of the genus *Macaca* of the Old World Cercopithecidae. Except for the Barbary macaque, they are of Asiatic distribution, ranging from Afghanistan to the Philippines, and from Java to 41° north latitude on the main island of Japan. From south to north, they demonstrate admirably the principle of reduction of extremities to conserve body heat. Thus the long-tailed macaque is found along the southern, extremely tropical periphery of the range in Malaysia. To laboratory people, it is known as the cynomolgus monkey. Other names for it are Philippine macaque, Java monkey, kra, and crab-eating macaque. To the north in India and south China we find the rhesus macaque, with a tail of medium length. Finally, in northern Burma, Indochina, China, and Japan, the large heavy-set, red-faced, or stump-tailed macaques are found. In these monkeys, the tails are reduced to ridiculously little stubs, measuring scarcely two inches in length. The Japanese macaque is extremely hardy, foraging about in the snowy pine forests to the northern part of Honshu Island, which approximates the 39° F. mean winter high isotherm. The mean winter low is 23°, but temperatures below zero Fahrenheit have been recorded.

There are at least 12 recognized species of macaques, indicating their successful position in the primate world. The rhesus macaque (*Macaca mulatta*) is, and will probably remain, the laboratory monkey par excellence. It thrives under adverse conditions in India, competing successfully with other primates, including its chief competitor, man. It can withstand the 100°+ temperatures of the Indian lowlands and the snows of Tibet at altitudes of 13,000 feet. It survives in cities and villages, and is not dependent on forests, for it is at home on the ground. In fact, the activities of man tend to encourage the rhesus and several other species of macaques. These monkeys might be compared to the white-tailed deer of eastern North America, which have increased tremendously since civilization altered the landscape and produced the bounty of farm lands and cut-over forests.

The macaques are basically monkeys of clearings, edges, stream banks, and second growth, whereas the leaf-eating langurs of Asia are more dependent on

unbroken forest. While a member of the George Vanderbilt Sumatran Expedition of 1939, I was surprised to find long-tailed macaques abundant on the outskirts of the capital city of Medan. They were exceedingly bold around the local zoo where we had our headquarters, and would descend to my open-air

FIGURE 3. The robust, heavily furred, and tailless Barbary "ape" (*Macaca inuus*) is an excellent example of a monkey modified to conserve body heat in a cool climate. It ranges to about 37° north latitude in North Africa. A closely related member of the Cercopithecidae, the Japanese red-faced macaque (*Macaca fuscata*) is the northernmost-ranging nonhuman primate, reaching 41° north latitude on the main Japanese island of Honshu.

work table and snatch scalpels and other tools the moment I walked away. They spent much time on the ground and raided my small mammal trap lines in a most annoying manner. So exceedingly common were they that the local zoo owner used them as feed for his lions and tigers.

At the time, I wrote[2]: "The crab-eating macaque is ubiquitous. We found the animal everywhere from the tidal flats of the Deli River up to 3000 feet in the cultivated valleys. In the mangrove swamps of the Deli River, small bands were seen on mud flats, feeding close to the water's edge. When alarmed,

these monkeys ran away across the mud, not resorting to trees as the langurs did under like circumstances. The cheek pouches of those that I collected were filled with large beans and pieces of bean-pods. Near Medan they haunted the plantations and native gardens. Along the railroad between Medan and Siantar, I noticed bands of them in the scrubby jungle that lay between the tracks and the big plantations. As the train passed, the animals would jump up and down excitedly. The natives often keep these monkeys for pets, as they also keep the larger *M. nemestrinus.* At Blangnanga, Atjeh, we had a tame young female whose chief occupation, when she was not catching flies, was turning over sods in search of beetle larvae."

I again encountered long-tailed macaques on the island of Nias, off Sumatra's West Coast—the dark-tailed form *Macaca irus phaeura*—and wrote: "The country around Hilisimaetano consisted of low rolling hills, on which were native gardens, large tracts of coarse, tall grass, and occasional patches of thick jungle in which a spiny, climbing palm (*Calamus*) was very common, and clumps of bamboo were plentiful. It was only in the more remote growths of jungle that the Nias macaques were encountered; but in such places the animals were fairly numerous, travelling in bands of about a dozen individuals. They were always high in the trees, where their typically macaquelike chucklings would be heard long before the animals themselves could be seen. In keeping so strictly to the trees, they differed notably from their Sumatran relatives, which appeared to spend much time on the ground."

The southward displacement of the long-tailed macaque in southeast Asia is in keeping with our experience in attempting to maintain them in a spacious outdoor colony at the Philadelphia Zoo. In 1955, unable to obtain rhesus monkeys, we decided to try *Macaca irus* on our Monkey Island instead. Of 43 placed on the island, 25 died—a mortality rate of 58 per cent. The largest single cause of death was pneumonia. As is too often the case, these monkeys arrived in a debilitated condition, and many of the deaths occurred in late spring when the weather was still cool. However, the significant fact is that no pneumonia occurred during the warm summer months, yet it reoccurred in September with cooler weather, despite the fact that the monkeys had had a fair amount of time to build up their resistance on our balanced diets. It is obvious that this species is very delicate where even moderately low temperatures are concerned.

By comparison, rhesus monkeys proved much hardier in the same situation, although their average annual mortality over a twelve-year period has been 23 per cent. This is due largely to the facts that they arrived in a debilitated condition and that we obtained a new lot every spring and disposed of them in the fall. In 1952, we decided to overwinter the colony on Monkey Island, with no protection except for two wooden doghouselike shelters in which they could crowd together for warmth. Hay in these shelters proved useless, for the monkeys pulled it out and scattered it. We lost just one animal—a female that fell through thin ice and died of exposure. To give some idea of how hardy well-conditioned rhesus monkeys are, I cite the case of another female that almost died in the same fashion. She broke through the ice and was trapped under it. The head keeper found her there, apparently lifeless, and was carrying her

up the walk by the hind legs, when I stopped my car to look at the animal. I detected a faint heartbeat, and rushed her to the laboratory, where we applied heat, rubbed her briskly with towels, and manipulated her limbs until she revived completely.

Because of the poor condition of the rhesus monkeys upon arrival and the high mortality that followed their immediate release on Monkey Island during the cool weather of early May, we now try to obtain them about six weeks in advance in order to condition them and treat them with antibiotics if necessary. The trauma of capture in India and shipment to the United States, coupled with protein starvation, leaves their resistance extremely low. When first released into detention cages in the conditioning room, they are extremely nervous and apprehensive, and the approach of a human being sends them rushing instinctively to the tops of their cages, as they would seek a tree in the wild. Since injuries may occur in these wild stampedes, we avoid disturbing them and have a single attendant care for them until they become better adjusted. Few deaths occur on Monkey Island to animals thus conditioned prior to their release. In recent years, the feeding of rhesus monkeys held in India and in transit has improved, but there is still room for improvement. Cebids are still arriving from tropical America in exhausted and malnourished condition.

The popular idea that monkeys are vegetarians requiring mainly carbohydrates is false. Unfortunately, this misconception has sometimes extended to the laboratory. Experience at the Philadelphia Zoo has shown that the high-protein diet cannot be stressed too highly, and by this we mean meat, for vegetable proteins do not fill the bill. Outstanding work has been done in the field of animal nutrition by Herbert L. Ratcliffe, of the Penrose Research Laboratory. The diet for omnivorous animals that Ratcliffe developed contains cooked ground horsemeat and meat broth mixed with cereals, minerals, and vitamins.

We also feed our monkeys raw ground horsemeat, which they eat with great gusto. On one occasion, I fed raw meat to our spider monkeys and they ate it ravenously, as though it were a great delicacy. Almost all monkeys are insectivorous, and I have observed guenons, mangabeys, and macaques catching and eating flies. However, in our experience the Cercopithecids can withstand protein starvation better than Cebids.

In 1954, in a popular article aimed at people who persist in keeping monkeys as pets, I wrote[3]: "Squirrel monkeys are highly insectivorous in the wild, and need plenty of meat in captivity. In fact, all pet monkeys need a high-protein diet, and this brings up a point of great annoyance to me. Time and again people have told me that the pet shop instructed them to give their monkey no meat because this would make it fierce and cause it to bite. This is a very erroneous, old-fashioned idea. Meat doesn't make monkeys fierce; it merely makes them healthier, and, of course, vigorous, healthy individuals are more apt to bite."

With the advent of healthy, vigorous, so-called "normal" monkeys, another factor becomes increasingly important. This is social pressure—the result of the social order, variously referred to as hierarchy, butt order, or hook order.

Social organization is complex, and plays an important part in the lives of wild primates. Close confinement and resultant crowding have a drastic effect on all monkeys. In close confinement, disturbances of social organization are intensified. In the Philadelphia Zoo, physical conflicts and deaths from injuries have greatly increased. Even where overt aggression is not present, animals low in the social order degenerate and eventually succumb to disease. In an article entitled "Changing Frequency of Arteriosclerosis in Mammals and Birds at the Philadelphia Zoological Garden,"[4] Ratcliffe and M. J. Cronin offered a possible explanation for the recent marked increase in this disease in various groups, including the Cercopithecidae and Cebidae. On page 47, they state: "The last rise in frequency occurred in association with increasing numbers of animals on exhibition, and an over-all rise in injury as a cause of death. Thus, it seems reasonable to suggest that improved diets have led to an increased frequency of arteriosclerosis by either of two mechanisms: increased life spans of relatively inactive animals, or increased vigor and reproductive drives. The second seems to have been the more important during the last decade of this study." Again, on page 49, they state: "Therefore, we suggest that social pressure has been a major factor in the recently increased frequency of arteriosclerosis in mammals and birds of the Philadelphia Zoo."

In an effort to reduce this mortality from injury and arteriosclerosis, we have sharply reduced the number of animals on exhibit and also the number of cages, although the new cages actually do not contain as many cubic feet of space as the old ones because they are lower. One group of monkeys disposed of was the bonnet macaque family—long-tailed macaques from South India. This was a breeding group of long standing, consisting usually of one large adult male, two breeding females, and several offspring in various stages of development. Their record was not good. The adult male was the Alpha animal, brutally aggressive and thriving at the expense of the rest. When he was unable to vent his aggressive tendencies on a passing keeper, he would turn upon the luckless females and juveniles in the cage. As a result, few youngsters reached maturity and head injuries were common, due to the babies' heads striking the bars when they were clinging to their mothers' breasts and these animals made wild leaps to escape the male's attacks. Prolapse of the rectum was also common, with several fatal cases. Such vicious attacks are also common among our baboons, and we have found it necessary to remove the young ones at an early age to ensure their survival.

Our groups now consist chiefly of just two animals—male and female—and, almost invariably, the male dominates. The time of greatest conflict is when food is introduced; frequently the male will gather it all around him so as to prevent the female from obtaining any. Cheek pouches do sometimes aid subordinate Cercopithecoids in carrying food off to a safe distance, where it can be eaten, but often I have observed Beta animals so impressed by the Alpha monkey and its threatened retaliation that they simply would not touch food lying close beside them.

Earnest Hooten, in *Man's Poor Relations*[5], claimed that New World monkeys are less greedy than those of the Old World because they lack cheek pouches. Actually, I feel that it is because they are far more arboreal and do not need

them. Pouches are necessary when monkeys descend to the ground to feed hurriedly, then ascend trees to eat in safety. Most of the Cercopithecoids spend a considerable amount of time on the ground. Monkeys have not taken to trees by choice, but of necessity. The most successful types are those that have developed a degree of independence from trees. This is why I consider the Cebus monkeys the most successful in the New World. Raymond Gilmore,[6] while studying yellow fever in Brazil, had this to say: "Cebus monkeys were the main catch, and were taken in quantity—126 in one locality in 2 years; 72 in another locality in 8 months. Many cebus were also caught in ground traps. The surprising ease with which this monkey was trapped was due to its abundance, inquisitiveness, and dominating aggressive behavior. When individuals were accustomed to man, as they were around the cornfields in the sparsely wooded areas of southern Brazil, they rarely showed hesitation in entering traps to rob the corn and banana bait, and as many as 3 have been caught, on several occasions, in the same trap at the same time."

Hooten also stated that New World monkeys are very low in dominance as compared to the "brutal" Old World types. Perhaps he was thinking in terms of howler monkeys, for this certainly is not true of Cebus and spider monkeys. In our experience, Cebus colonies show strong dominance patterns and, while the dominance of spider monkeys is more subtle, it is very far from low. For example, during the last three years we have tried to maintain a cage of mixed Cebids—spiders, woollies, and capuchin monkeys—without success. Today the spiders are the sole survivors. The woolly monkeys did well until introduced into this mixed group, when they declined rapidly and died. The capuchin was the next to go, although she was removed some time before her death when it was obvious that she lived in great fear of the spider monkeys, despite the fact that they did not openly molest her. The dominant animal in this group was a female Colombian brown spider monkey (*Ateles belzebuth hybridus*).

Conflicts within a single species group have less serious results in our experience than those between species. Due to lack of space, I tried three species of African guenons together in 1957—a male Campbell's monkey, a female Diana monkey, and a female L'Hoest's monkey. The two females were dead in less than six months, and the Campbell's monkey survived less than a year.

An investigator studying the yellow fever virus recently asked for advice in maintaining howling monkeys in the laboratory. Our experience has been very poor. The animals invariably arrived in a debilitated condition and showed little interest in food, despite our strenuous efforts to provide them with every possible form of nutrient. We even went so far as to prepare a semi-liquid mixture and to force-feed them, without success. The monkeys simply huddled disconsolately in a corner and grew weaker and weaker until they died. It is possible that the red howlers (*Alouatta seniculus*) we received from commercial sources were degenerate specimens to start with, such as are described by Hershkovitz,[7] who writes: "Many series of red howlers taken by the writer appeared to be remnants of erstwhile large populations, confined to small relicts, or facsimiles thereof, of primary forest. In these delimited and altered habitats individual clans of howlers have become isolated from the mass of the popula-

tion. Individuals persist as long as nature provides them with a suitable tree and its fruit. Unlike their more resourceful, or more adaptative relatives, howlers do not ordinarily supplement their ever-diminishing natural larder with loot from cultivated fields circumscribing their domain. They simply eat less and travel less. Their growth becomes stunted, their resistance to disease and parasites reduced." The way to develop a laboratory colony of howlers, I believe, is to make a special trip for them and collect only young animals that would be more amenable to an artificial diet.

According to the observations of Carpenter,[8] Collias and Southwick,[9] and Altmann,[10] intraspecific strife is very low among howling monkeys. There is little competition over food. Since they feed largely on leaves, they may be compared to cattle grazing in a field. Schein and Fohrman,[11] in their study of social dominance in dairy cattle, have this to say: "There is little doubt that the lower order animal would suffer markedly if she were wholly dependent on trough feeding. Apparently 'space' is the environmental factor in short supply (thereby stimulating competition) in barn hay feeding, since no spatial competition is evident in open pasture grazing." Certainly space is the factor usually lacking in both zoo and laboratory primate colonies. Comparatively free-ranging colonies in large, outdoor enclosures have proved far healthier. For better results, monkeys should be thus conditioned for periods of at least six months before being used for research.

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CLIMATIC CONDITIONS IN THE NATURAL ENVIRONMENT OF MONKEYS

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As a meteorologist I have been interested in many aspects of weather and climate. Some of my investigations have led me into a consideration of the effects of weather on the health and behavior of human beings. The question of interrelation between human health and behavior and the dynamic forces of weather and climate in temperate zones is presently wide open. About a year ago a group of scientists banded together to consider methods of attacking problems in medical climatology. This led to the formation of the American Institute of Medical Climatology, a nonprofit organization now in the process of incorporation under the laws of the Commonwealth of Pennsylvania.

One of the earliest workers in the field of climate and health, Clarence A. Mills (Mills, 1959), points out that climate is now recognized as a major factor in human health and disease, dominating the dynamics of life from conception to death. In some regions, the climate is of such a nature that it generates positive good health and a high level of vitality. In other areas the climatic environment results in a passive lethargy only slightly above the vegetative level, and this applies to both laboratory and domestic animals, as well as to human beings. Certainly, much more should be known about the degree and direction of the effect of the physical environment on such things as the growth and development, the reproductive functions, the energy level, the resistance to infection, and the rate of body breakdown of humans and animals. Only in recent years have physicians come to see how promptly and how strikingly men and animals respond to changes in physical environment, especially with changes that alter the ease of body heat loss. For example, Mills points out that growth is affected by body heat loss to such a degree that high external temperatures lead to growth retardation. In Iowa, a steer is brought to 1000-lb. choice slaughter size at 15 months of age. In Louisiana, this requires 2½ to 3 years; in Panama, 5 years. Furthermore, this is top adult weight for tropical animals, but in temperate climates they can reach twice this size in time. The climatic factors that seem to affect man most are high temperature and high humidity with little diurnal or seasonal variation. As a result of such conditions the body functions receive very little stimulation, heat dissipation is difficult, and energy expenditure is low.

My first impulse on undertaking this paper was to acquire as much literature as possible on monkeys and their habitat and diseases, and then try to slant this discussion toward possible effects of changing climates on the health and growth of monkeys transplanted from their area of origin. After a little thought, however, it was clear that this was not my intended goal; I therefore present, instead, a few ideas embracing climate in general, and the specific climate of the chief source regions of research monkeys.

The weather at a given place is the sum total of all of the meterological elements, including temperature, pressure, relative humidity, wind velocity,

precipitation, and cloudiness. Even though changes in the weather are many and varied, it is possible to arrive at a composite picture of the weather by averaging these variations. Such a generalization is called the climate of an area. However, the climate of an area is not determined solely by the long-term annual averages of the meteorological elements. Edinburgh, Scotland and Boston, Mass. have nearly the same annual average temperature, about 48° F. However, the temperature extremes to which they are subjected during the year are markedly different. The variations at Edinburgh range from 38° to 58°, while at Boston the range is from about 27° to 70°. Thus, to characterize the climate, it is necessary to consider also the regular variations to which the meteorological elements are subjected, particularly seasonal changes. Temperature, of course, is not the only element to be considered. Cairo, Egypt and New Orleans, La. have about the same mean temperature (68° F.) and similar variations, but an annual rainfall of 1.3 inches at Cairo compared to 56.5 inches at New Orleans makes their climates quite different. Therefore, the climate of an area is described by the mean state of the atmosphere at a given place and the variations to which the mean state is subjected.

Since the atmosphere is essentially a heat engine run by radiant energy from the sun and since the condition of the body is often governed by heat absorption and heat loss, temperature is perhaps the most important of the climatic elements. Because the heat-loss process is dependent on rate of evaporation, and because this is a function of relative humidity, this element is also a climatic factor of importance in this discussion. Precipitation is probably next to these in importance as a climatic element.

Formal classification of climates is generally aimed at picturing climatic effects on animal and plant life. Temperature and precipitation are the two principal elements in most classifications because heat and water are the two most important factors in the organic and inorganic world, and these are the two variables most regularly and generally observed. Of course, elements such as evaporation, ground temperature, radiation, and winds are also important, but the distribution of these is reflected in the distribution of temperature and precipitation if these two elements are suitably combined.

The most widely used classification of climates is one developed by Köppen (Köppen and Geiger, 1938). It attempts to combine the climatic elements and their effects on animal and plant life. Köppen chose certain numerical values of temperature and precipitation to serve as criteria for determining the boundaries between different types of climates. These numerical values were selected according to their effect on plant growth because the vegetation cover of the earth is of the greatest economic importance to civilization. It is possible to combine temperature and precipitation data to find expressions permitting the grouping together of regions with the same climatic effects and allowing us to distinguish between regions where the effects on plant, animal, and human life are different.

To obtain a sufficiently detailed description of the different climates that allows simple grouping, Köppen uses only five main divisions, each designated by one of the first five letters of the alphabet in capitalized form. These main divisions are subdivided, each subdivision being designated by other small or

capitalized letters of the alphabet. Thus, a highly descriptive "climate formula" can be obtained that indicates temperatures and precipitation and the seasonal trend of these two elements.

For plant growth in temperate latitudes, the heat during the summer vegetation period is more important than the temperature during the winter. At lower latitudes a season of dryness has an effect similar to a season of cold weather at higher latitudes. It is important to know whether the temperature remains below freezing during the entire winter or not. If so, even slight snowfall is sufficient for plant growth in the spring. If dry and cold seasons vary and if part of the year is dry, the conditions for plant life are entirely different, depending on whether precipitation falls during the winter or during the summer.

On the basis of such considerations, Köppen set up climatic types according to those shown in TABLE 1.

These climates are separated from each other by the values of the extremes of the monthly mean temperatures. As the boundary between the region of

TABLE 1
KÖPPEN'S CLIMATIC CLASSIFICATIONS

Climatic group		Dry period	Degree of dryness and cold
Tropical rainy	A	f s w	
Dry	B		S W
Warm temperate rainy	C	f s w	
Cold snowy forest	D	f s w	
Polar	E		T F

trees ACD (humid climates) and the polar climates E, the temperature of 50° F. during the warmest month is chosen. Where the temperature of the warmest month is below 50° F., the polar climate E prevails. The 50° F. temperature during the warmest month is used because it coincides approximately with the poleward limit of forests.

The limits between the climates A, C, and D are determined by the mean temperature of the coldest month. Where the temperature of the coldest month is above 64.4° F., the area falls into the tropical rainy type of climate (A). If the temperature is between 26.6° F. and 64.4° F., the climate type is C and, when the temperature of the coldest month is less than 26.6° F., the climate falls into category D. A temperature of 32° F. for the warmest month separates the tundra climate (ET) from the climate of permanent frost (EF). The limit of 64.4° F. was chosen as the lower limit for the A climates because this temperature is considered optimum for human comfort. The small letters *f*, *s*, and *w* indicate the absence of a dry period (*f*) or the presence of a dry period in summer (*s*) or in winter (*w*). It might be expected that tropical rain forests with their high humidities would not exist if a regular dry season prevailed. However, precipitation during the other months may be so great that sufficient moisture is retained in the ground to maintain the tropical forest during the drier months. This type of climate is denoted by Am (monsoon). It is

intermediate between Af and Aw, resembling Af in amount of precipitation and Aw in seasonal distribution. If the amount of precipitation during the driest month is greater than 2.4 inches, the climate is classified as Af. Based on this classification, areas of the earth fall into 11 principal types of climate.

In characterizing climatic conditions in the natural environment of monkeys, a look at a climatic map of the earth simplifies our discussion. It is evident that most of the Philippines, Malaya, Burma, Indochina, India, and the section of Africa between 15° N latitude and 15° S latitude fall into the A type of climate, and predominantly the Aw type. These areas, in turn, constitute the principal source regions of the research monkey.

The temperature of these climatic types is above 64.4° F., even during the coldest month. Thus, there is no winter in the natural habitat of the monkey. The mean annual temperature is between 75° F. and 85° F. The annual range of temperature is small, considerably less than the daily variation. For example, the day to night variation in temperature may be 15° F. to 20° F., while the difference in average monthly temperatures may be no more than 2° F. to 5° F. Also, in the A climates there are no aperiodic changes in temperature, so the mean temperature of the same months during different years is the same.

TABLE 2

ELEMENTS OF AN AF-TYPE CLIMATE

	J	F	M	A	M	J	J	A	S	O	N	D	Yr.
Temp. (°F.)	78.3	79.0	80.2	80.8	81.5	81.1	81.0	80.6	80.4	80.1	79.3	78.6	80.1
Precip. (in.)	8.5	6.1	6.5	6.9	7.2	6.7	6.8	8.5	7.1	8.2	10.0	10.4	92.9
R.H. (%)	83	80	81	84	83	83	83	84	84	84	85	84	83

TABLE 3

ELEMENTS OF AN AW-TYPE CLIMATE

	J	F	M	A	M	J	J	A	S	O	N	D	Yr.
Temp. (°F.)	66.6	71.2	80.2	85.5	86.2	85.2	83.7	83.1	83.1	79.4	73.6	66.6	78.7
Precip. (in.)	0.4	1.1	1.4	2.0	5.0	11.2	12.1	11.5	9.0	4.3	0.5	0.2	58.8
Max. temp.	79	82	90	92	91	88	88	88	89	88	84	79	86
Min. temp.	53	58	67	73	75	77	77	77	77	73	64	55	69
8 a.m. R.H.	79	76	75	74	77	83	86	86	82	78	78	80	79
5 p.m. R.H.	58	54	51	56	73	82	83	81	81	78	73	69	70

Rainfall is abundant, rarely less than 30 inches per year, and usually much more. Much of the rain is convectional in nature, depending largely on intensity of solar heat received, so most of the rain occurs at certain fixed times of the day, mostly between noon and midnight. Amount of rainfall is more variable than temperature. It shows changes with the season and with the locality, and aperiodic changes from year to year.

Data from Malaya serve to illustrate conditions characteristic of an Af-type climate. In TABLE 2, temperature and precipitation figures are for Singapore, while the average relative humidities apply to nearby Malacca, Malaya.

The Aw-type climate can be illustrated by similar data for Calcutta, India, and Comilla, Pakistan. For Calcutta, the mean monthly values for temperature and precipitation are shown in the first two lines of TABLE 3. Here, the monthly mean temperatures range over almost 20° F. compared to about 3° F. for Singapore, and the rainfall regime indicates a winter dry period. The humidity range in this type of climate is also wider and, in general, the relative humidity is somewhat lower. There is a wider range in daily temperature variations, too, both of these being illustrated with data for Comilla in the last four lines of TABLE 3.

In general, monkeys in their native habitat live in climates that are hot, humid, and rainy. The average temperature of the coldest month is greater than 64° F., and absolute minimum temperatures very rarely go below 50° F. Daytime temperatures are generally in the 80s and 90s. Relative humidities are quite high, averaging over 70 per cent and rarely falling below 50 per cent.

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PROBLEMS ASSOCIATED WITH THE TRANSPORTATION OF MONKEYS

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The transportation of monkeys from a wild state in the Orient to a captive one in the United States is obviously not a simple event that may or may not effect the health of the animals, but is rather a series of events varying a great deal in duration, circumstances, and consequences. It is safe to say that each event of the series probably has some effect on the health of each individual animal but, because it is difficult if not impossible to isolate transportation from concurrent environmental conditions, proper evaluation of the extent of that effect can only be presumed at this time.

Factors of transportation may influence monkey health in so far as they contribute to an unsatisfactory situation in the physical environment of the animals or a poor sanitary condition of their housing, food, and water, or as they introduce stress factors that directly or indirectly affect the health status of the animals.

Transportation details as discussed in this paper apply only to the handling of *Macaca mulatta* from northern India.* Although many of the observations may also apply to other species, or to rhesus monkeys handled by other agents, no attempt has been made to account for similarities or differences. Related environmental factors paralleling the various stages of transportation are reviewed briefly in so far as they are pertinent to the transportation picture.

From the time the trap snaps shut on a wild rhesus monkey until he is used as a research animal or in the testing or production of poliomyelitis vaccine, he will encounter eight or more separate transportation situations, which include: (1) from trap to trapper's headquarters; (2) from trapper's headquarters to a Vita buying station at Lucknow, Shahjahanpur, or Kanpur; (3) from buying station to local railway station; (4) from local railway station to Delhi railway station; (5) from Delhi station to Vita farm; (6) from Vita farm to Delhi airport; (7) air transport from Delhi to Beirut, Lebanon, or Bahrain to Amsterdam, Holland, or London, England, to Shannon, Ireland, to Gander, Newfoundland, to Indianapolis, Ind.; (8) from Indianapolis airport to Eli Lilly and Company in Indianapolis, either at (a) the McCarty Street facility, where they are conditioned and used as test animals or in the production of poliomyelitis vaccine, or at (b) the Greenfield farm, where some animals are conditioned; and (9) from Greenfield farm to the McCarty Street facility.

Animals that are not shipped directly to the ultimate consumer in the United States must encounter one or more additional shipments within this country.

Even though the monkey herds are a constant menace to their meager food supply, the villagers are often reluctant to see the animals captured. Consequently, the trapper is apt to hide the newly trapped monkey in almost any type of container, including even the folds of his clothing. The monkey may remained in this improvised container the remainder of the day, but usually

* Through Vita Private, Ltd., Delhi, India, to Eli Lilly and Company, Indianapolis, Ind.

he is soon placed in a small bamboo crate with other animals captured that day, and so is transported to the trapper's headquarters. This cage is never cleaned or sanitized, and is replaced with a new one only when it is no longer serviceable. Thus, the newly trapped monkey encounters his first experience with the excretions and secretions of monkeys outside his own family group. Food and water are not usually given to the animals until they arrive at the trapper's headquarters that night. Therefore, sanitation in that respect is not ordinarily of immediate concern.

At the trapper's headquarters the newly captured monkeys are placed in larger bamboo slat crates measuring 36 by 60 by 30 inches high. Twenty animals are normally kept in this crate. Water is supplied in a large crock, and feed grain is sprinkled on the ground or floor beneath the crate. Monkeys pick up the grain through the spaces between the slats. Contamination of the grain with waste materials is obvious, and each animal directly contacts secretions and excretions from a few more animal contacts. Both these factors are significant health problems, along with the fact that the diet frequently is inadequate both quantitatively and qualitatively.

Under optimum conditions the animals are soon offered for sale at one of the local buying stations. Each animal is examined and, when bought, is usually transferred to a new crate of similar design. If the animals are held on speculation by the trapper or an independent agent, highly unsanitary conditions are apt to be experienced, and a serious health hazard results.

Monkeys are held at the local buying stations only a few hours. Trains run to Delhi each night, so the animals are generally shipped the same day they are bought. The crates are transported to the railway station by wagon, truck, rickshaw, or other local conveyance. The animals are fed and watered at the station just before loading on the train.

The bamboo crates are loaded aboard small, enclosed freight cars for the twelve- to fifteen-hour trip to Delhi. Railroad regulations require that the side doors be kept closed and locked. Consequently, a ventilation problem may arise in extremely hot weather or if the car is badly overcrowded. However, one might logically expect that any health problem arising from this situation would be immediately recognized as such. The fact that shipments are made at night helps. No food or water is given en route.

At the Delhi railroad station the animals are unloaded from the rail cars, given a gross examination by a veterinarian, and immediately put on an open truck for transport to the Vita farm. Although a large tarpaulin is thrown over the box of the truck during rainy weather, the animals could get wet or chilled.

At the Vita farm the animals are examined, sorted, and assigned to large, roomlike pens, holding from 100 to 300 animals. Thus, they are brought into direct contact with many more animals from even more widely separated areas. The diet is excellent, as the animals are given adequate amounts of a mixed-grain ration (equal parts of rice, barley, and gram) twice each day, and an in-season fresh fruit or vegetable once each day. Considering the personal habits of the monkeys and the large numbers of animals involved, the sanitation and care provided at the farm are also very good. Each cage is cleaned twice each

day and sanitized once each week. Animals generally remain at the farm for from 2 to 10 days while a planeload of 1800 animals is being assembled.

When the monkeys have been examined and selected for shipment to the United States they are packed into a wooden shipping crate measuring 21 by 36 by 19 inches. Seven large or nine small monkeys are placed in each crate. The loaded crates are transported to the Delhi airport on open trucks under conditions similar to those previously discussed. However, the consequences of having the animals get wet while being transported to the airport or while being loaded aboard the aircraft are potentially more serious at this stage.

Once aboard the aircraft en route to the United States, monkeys seem to travel well if they are comfortable. The metabolism and waste products of 1800 monkeys put a considerable strain on an airplane ventilation system designed to keep 80 or 100 human passengers comfortable. Further complications arise from the fact that the plane travels through and stops at climatic areas ranging from tropical to arctic. Consequently, potential problems of temperature, humidity, and air movement are continuously presented. Uncomfortably high cabin temperatures are commonplace while loading the plane at Delhi or during service stops at Karachi, Bahrain, or Beirut. From that point on, cold and damp climatic conditions are apt to be more troublesome than is excessive heat. Very inclement weather may be experienced during service stops at Amsterdam, London, Shannon, and Gander. While the plane is in the air, cabin temperature is a relatively minor consideration, as the outside air temperature at an eight- or ten-thousand-feet altitude is never excessive, and most planes have effective cabin heaters to use when necessary. Whenever the plane lands and the engines stop, the heating, cooling, and ventilating equipment on the plane also stops, however; and it is during these times that most of the potential climatic problems arise. The cabin doors and windows are usually opened wide during the service stops in order to compensate for the lack of ventilation. Thus, a great deal of cold air can at times pour in on the animals in the crates on the floor of the plane.

The movement of air through the cabin of the aircraft was considered a potential health factor when the DC-4 aircraft was generally used to transport monkeys. Even though the temperature of this moving air was well within the normal comfort range, the physical effect of the draft itself seems to be conducive to animal discomfort. Heat, humidity, and air movements are all factors that we feel have been significantly improved by transporting monkeys with pressurized equipment such as that in the Lockheed Constellation or Douglas DC-6B.

Some monkeys show evidence of motion sickness while in flight. It is doubtful, however, that this phenomenon has any pertinent relationship to the complex animal disease picture resulting in death of some of the animals.

Sanitation within the shipping crates leaves much to be desired. Each crate is new and clean at the beginning of the journey, however. Wooden slats with 1½-inch spaces between them comprise the floors of the crates. Inserted an inch or two beneath these slats is a metal tray intended to catch the waste materials. These pans are cleaned at approximately 24-hour intervals. Of course, the slats are contaminated as waste materials drop on and between them.

Whole-grain food is given to the animals in shallow trays, which slide into the shipping crate at floor level, at approximately 8-hour intervals. The food is soon contaminated by the monkeys, but since the caretakers clean the food trays between feedings, this sanitation problem is dealt with to some extent. A great deal of grain is spilled out of the food trays onto the floor trays, however, where it is grossly contaminated with monkey waste. Unfortunately, monkeys habitually eat this contaminated grain between regular feedings.

Water containers, which consist of tea tins wired securely in the front corners of the crates, cannot be removed for cleaning. Consequently, fresh water is periodically added to the already contaminated water in the cans. Monkeys often refuse the contaminated water, but manifest thirst as fresh water is being added. Water is usually taken aboard the aircraft at each regular service stop. Thus, the physical character of the water may vary a great deal during the course of the flight. This fact alone can upset the digestive system, even when traveling within the United States.

Many people have been interested in the diet of the monkeys during transportation as well as following that period. Some have thought that a quantitative or qualitative deficiency might be responsible for the difficulties that are sometimes experienced. It is my opinion that there is a greater hazard to the gastrointestinal tract of the animal in free-choice feeding of heavy concentrates than in giving a small quantity of light food at infrequent intervals. Certainly, a concentrate such as corn has no place in this situation. Changing the physical character of the food from whole grain to a biscuit form would greatly improve food sanitation within the shipping crate.

Upon arrival in Indianapolis, the monkeys are immediately transferred from the aircraft through an enclosure to enclosed company-owned trucks, and they are then transported to one of Lilly's monkey facilities. In very inclement weather, it is possible that the animals may be chilled in making this transfer. This operation has been considerably improved as animal-handling experience has been gained. It is done as expeditiously as possible.

Thus far little mention has been made of that much used and abused term, stress, despite the fact that it must almost certainly be of some consequence in monkey transportation problems. Certainly everyone recognizes the fact that monkeys are wild, excitable animals that resent confinement and respond excessively to excitement stimuli. The inherent characteristics of the species may make them capable of magnifying stress factors directly into significant animal health problems. For the most part, however, stress factors are of interest because of the influence they may have upon the relationship of the susceptible host to other disease factors. Again, it is difficult, if not impossible, to differentiate between the effects of stress and other concurrent disease factors until more scientific evidence is available.

Circumstances that are conducive to the development of stress factors occur at each stage of the transportation series. The first and probably foremost psychologically is that of suddenly losing all freedom in the trap and cage, as well as a sudden separation from mother, family, and familiar surroundings. Complete adaptability of each individual is a necessity, as there is no attempt to compensate for these losses. Additional stress factors are introduced whenever the animals are caught, handled, or put into a cage with unfamiliar com-

panions. Each time the latter situation occurs it seems that it is necessary to establish an order of dominance with its resulting harassment of some individuals.

Stress factors due to extrinsic influences include the environmental conditions previously discussed.

Two rather cursory experiments conducted during 1958 and 1959 seemingly minimize the effects of stress factors during transportation and, indeed, minimize the effects that transportation in general may have on monkey herd health. In the first experiment, animals in one half of the crates aboard an aircraft were given a tranquilizer in their food, while the other half served as controls. There was no difference in death loss in the 2 groups either during transportation or during the following 2-week period. In fairness to the experiment, it should be pointed out that the animals had been held at the Vita farm in Delhi for from 2 to 4 weeks prior to shipment and that, en route, a delay due to engine trouble complicated the situation. In the other experiment, a sizable group of monkeys was selected as for a regular shipment, but was returned to cages at the agent's farm for a 60-day conditioning period rather than being shipped immediately. The death loss in this group was very comparable to what one might expect if it had been shipped immediately and then held for a 60-day period in the United States. The survivors of the group were subsequently shipped at the end of the conditioning period, with no disease problem evident.

The monkey health problems associated with procurement and transportation are obviously complex and, as such, are not apt to have a simple solution. Genuine efforts to improve conditions have been made by the transportation companies, the Indian government, and the suppliers. The fact that these efforts have not been completely successful may be due to the applicability of the old adage, "A chain is no stronger than its weakest link." Complicating the situation is the apparent fact that any break in the disease-prevention chain is not immediately discernible, but becomes evident only after several days or weeks, when the underlying factors of disease have been left far behind. Thus, an investigator is easily deluded by a face-value assessment of the monkey disease as he sees it.

Specific improvements can be made at each stage of monkey procurement and transportation. Most of these improvements are self-evident, but are also extremely difficult to implement. Furthermore, there is little assurance that any will be effective unless all the factors can be dealt with simultaneously and completely. Indeed, a dim view of the value of additional intensive efforts to control disease might be justified except for the fact that all rhesus monkeys appear to be in overt good health at the time they are captured and after they have survived the first two or three months of their captivity. Suggested disease-control efforts include the following procedures:

The study of monkey procurement and transportation methods should be continued, with an accumulation and application of experimental scientific evidence.

Newly caught monkeys should be placed in new or sterilized cages only. Each subsequent transfer should also be only to new or sterilized cages or crates.

Contact between monkeys should be avoided insofar as possible. At no time should any animal be in direct contact with more than two or three others. Contacts should have originated from the same geographical area, preferably from the same family.

Sanitation in shipping crates should be improved by (1) providing a cage floor that can be cleaned periodically, perhaps by using wood shavings or some type of replaceable paper cage liner; (2) providing removable water containers that can be cleaned periodically; and (3) changing the food-particle size from grain to that of a biscuit and eliminating corn and heavy concentrates from the diet during transportation.

Aircraft ventilation systems should be revised to provide the same ventilation conditions within the aircraft while it is on the ground as during flight.

All stages of transportation should be accomplished as expeditiously as possible.

Summary

The transportation of monkeys from a wild state in India to a captive one in the United States is a series of events that may adversely affect the health of the animals. This influence is shown by the fact that it produces an unsatisfactory physical environment, unsanitary housing, food, or water conditions, or introduces stress factors that influence the normal host-parasite relationship.

Closely related environmental conditions that occur concurrently with transportation are also important and difficult to differentiate.

Changing from unpressurized to pressurized aircraft for monkey transportation has improved many problems of physical environment. Aircraft ventilation, while on the ground, needs to be improved.

Sanitation of cages, food, and water needs improvement at each phase of monkey transportation.

Improved isolation of monkeys originating from widely separated areas would also improve the health status of the animals.

COLONY HUSBANDRY OF RESEARCH MONKEYS*

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The purpose of this paper is to provide a source of information for those interested in husbandry of laboratory monkeys and to disseminate data obtained through active participation with a large established monkey colony. Procedures and information presented reflect actual experiences witnessed in our daily routine.

The primate colony was established in 1951 by the School of Aviation Medicine, U.S.A.F., and the University of Texas as a part of the University's Balcones Research Center at Austin. Since that time about 3000 monkeys (*Macaca mulatta*) have been purchased and utilized in biomedical and psychological experiments. Colony strength is routinely maintained at approximately 700 monkeys, of which 500 are individually housed.

Colony Management

Many of the monkeys are on longevity studies and will be maintained in the colony for the remainder of their lives. A number of the permanent colony animals are approaching nine years of age and weigh 25 to 30 lbs.

The first problem encountered when dealing with the monkey is restraint. Monkeys over three years of age develop large canine teeth that make them more aggressive and, consequently, more dangerous and difficult to restrain. Two well-trained handlers can usually control one of these animals. Personnel handling monkeys wear double leather gloves with leather armguards to protect them against bites and scratches. Using this method of protection, the handler puts his hand in the cage, daring the monkey to attack. When he grasps the animal by one arm and lifts him from the cage, backward pressure is placed on both arms to control the animal. Except with very vicious animals, this procedure is easy for the handler and causes minimal stress to the animal.

A carrying cage is frequently used for moving animals. This cage is small, approximately one foot cube, with a sliding door on one end. After minimal training the monkey will learn to run into the carrying cage when it is placed against the home cage. Using this method, the monkey is not handled manually; this allows for faster handling and less trauma.

For the larger, more difficult-to-handle monkey, the squeeze cage is used. The monkey is forced against a sliding door and the appendages are made available for parenteral injections. The sliding door also provides a means of removing the animal from the squeeze cage.

The use of nets to restrain the monkey is a safe procedure, but it is a slow and cumbersome process.

Often it is desired to provide an animal in a semianesthetized condition to facilitate procedures such as ophthalmic examinations, T. B. testing in larger

* The work described in this paper was supported in part by funds provided under Contract AF 41(657)-149 with the U.S.A.F. School of Aviation Medicine and the Radiobiological Laboratory, U.S.A.F. Aerospace Medical Center (ATC), Brooks Air Force Base, Texas.

FIGURE 1. Cage used for carrying monkeys from one cage to another.

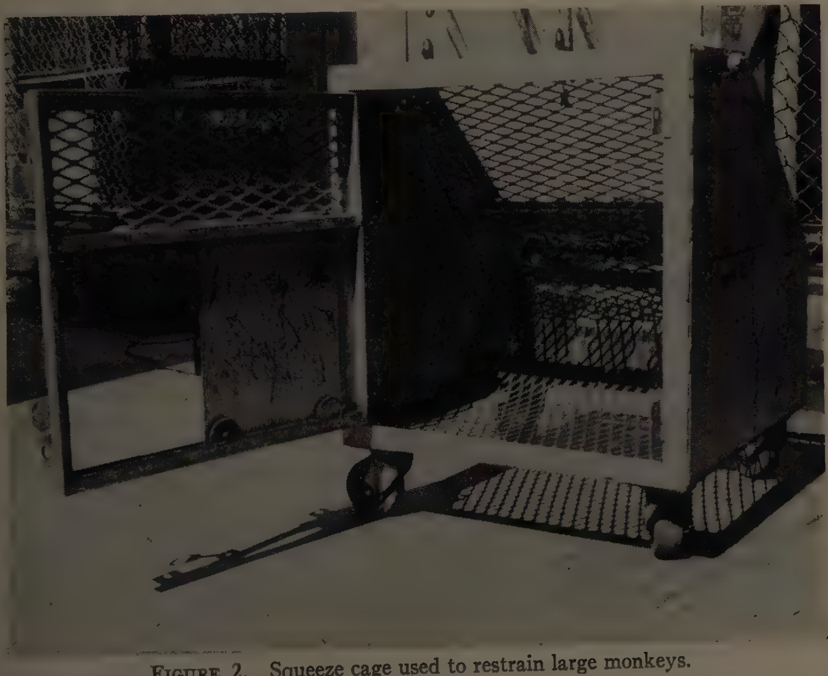

FIGURE 2. Squeeze cage used to restrain large monkeys.

monkeys, minor surgical practices, and weighing. For these procedures tranquilizing drugs are employed. Monkeys respond to tranquilizers in a uniform manner and tolerate these drugs well. Oral preparations may be added to the food ball with satisfactory results. Parenteral administration is usually preferred. Chlorpromazine (Thorazine) is administered intramuscularly at the rate of 0.65 mg./lb. of body weight and reaches maximum drug effect in 1 to 2 hours. The intravenous route requires one half this dose and becomes effective immediately.

Table 3 of optimal doses for barbiturates has been a valuable guide and timesaver when administering anesthetic agents to the monkey. Fast-acting injectables require a minimal number of technical assistants and equipment for administration. Inhalant anesthetics are well tolerated, but are used only when their certain advantages are specifically indicated.

Table 1

Normal Physiological Data Compiled and Used in This Laboratory[3]

Average Age of Animals: 2½ to 3 Years Old

	Mean	Range
Heart rate	193	180 to 220
Respiratory rate	55	45 to 60
Temperature (°F.)	103	102.5 to 103.5
Red cells/mm.^{3}	6.5 million	4.5 to 8 million
White cells/mm.^{3}	15,000	9000 to 18,000
Hemoglobin (gm./100 cc.)	12.6	10.0 to 16.0
Hematocrit (citrated)	41	32 to 52
Polymorphonuclear leukocytes per 100 cells	35	4 to 91
Lymphocytes per 100 cells	60	7 to 95
Monocytes per 100 cells	0.7	0 to 8
Eosinophils per 100 cells	2.0	0 to 5
Basophils per 100 cells	0.2	0 to 4
Sedimentation rate (per hour)	0.9	0 to 28
Plasma prothrombin time (sec.)	13	10.5 to 18.5

Synthetic opiates are occasionally indicated and have been utilized with highly satisfactory results. An average dose of meperidine hydrochloride (Demerol) is 12 mg., repeated as indicated. This dosage is usually adequate for the 8- to 20-lb. monkey.

The rhesus monkey is extremely nervous and energetic and is difficult to house. Unquestionably, animals involved in experiments should be housed in individual cages. The colony is housed indoors. All monkeys are in individual cages. Animal rooms are approximately 27 by 18 feet with 9-foot ceilings. The floors are concrete; the walls are either concrete blocks or glazed tile. This space is adequate to house 4 racks, each of which holds 10 individual cages. The cages in use were designed in this laboratory. These units have an overall length of 11 feet and a height of 78 inches. Cages contained in each unit measure 2 by 2 by 2 feet. All of these cages are constructed of corrosion-resistant aluminum 6061 alloy. Punched aluminum panels, welded construction, and a large, swinging front door are factors that facilitate cleaning the cage

thoroughly. Within the large hinged door is a small sliding door for removing the animal.

The temperature is thermostatically controlled and maintained at 68 to 72° F. A ventilating system that will maintain a constant temperature and humidity and provide circulation of fresh air is recommended.

Water is supplied by means of a pipe running behind each cage. A pneumatic quick-release coupling attaches the supply to the cage outlet. This allows the cage to be removed from the rack without disturbing the water system. Water is turned on twice daily for 30 min. Pans under the cage catch the excess water and drain it away.

TABLE 2
COMMONLY USED DRUGS AND DOSAGE

Aureomycin (chlortetracycline HCl)	250 mg. daily for 5 to 7 days, orally.
Carbarsone (*p*-ureidobenzenearsonic acid)	250 mg. daily for 10 days, orally.
Chloromycetin (chloramphenicol)	250 mg. daily for 5 to 7 days, orally.
Chlordane	0.3 per cent dip.
Delvex and Dizan (dithiazanine iodide)	10 mg. per lb. for 10 to 14 days, orally.
Demerol (merepidine hydrochloride)	1 to 2 mg. per lb. body weight, I.M.
Diodoquin (diiodohydroxyquinoline)	650 mg. daily for 10 to 20 days, orally.
Entromycin (bacitracin methylene Disalicylate)	6 gm. b.i.d. via stomach tube/feed.
Gentian violet	60 mg. daily for 14 to 21 days, orally.
Hexylresorcinol	0.2 gm.
Isoniazid (isonicotinyl acid hydrazide)	20 mg./kg. daily mixed in feed (prophylactic dose).
Metrazol (pentylenetetrazol)	¼ to ½ cc., I.M.
Milibus and aralen (bismuth glycolyl arsanilate) (chloroquine diphosphate)	1 tablet b.i.d. for 10 days, orally.
Morphine sulfate	¼ grain, I.M.
Nemural (4-oxy-3-actylaminophenyl arsonic-acid)	18 mg. tablet per 8 to 10 lbs. body weight.
Penicillin	300,000 units daily for 5 to 7 days, I.M.
Phenothiazine	60 mg. per lb. daily for 7 to 21 days, orally.
Serpasil (*Rauwolfia serpentina*)	½ to 1 mg./kg., I.M.
Sparine (promazine hydrochloride)	4 to 6 mg./kg., I.M.
Thorazine (chlorpromazine)	140 to 165 mg./kg., I.M.
Terramycin (oxytetracycline hydrochloride)	250 mg. daily for 5 to 7 days, orally.
Vermiplex (di-phenthane-70 and methylbenzene)	One No. 1 capsule per 5 lb. body weight.

Few animals respond so quickly to their diet as the monkey. Consequently, an adequate diet is of paramount importance. To ensure the desired ration a guaranteed-analysis feed is used. The following diet has proved very satisfactory for the animals at this laboratory. The figures listed below are the approximate amounts for a 6- to 12-lb. monkey.

⅓ lb.	Meal	(Monday through Friday)
1	Orange	(Tuesday)
1	Apple	(Thursday)
3	Chimcrackers	(Saturday and Sunday)

The meal is fortified with commercial vitamin preparations and mixed with water in a large electric food mixer. It is then manually formed into balls

TABLE 3

DOSAGE CHART:* PENTOBARBITAL SODIUM (NEMBUTAL)†

Weight of animal (lbs.)	Weight of animal (kgs.)	8 mg./lb.	10 mg./lb.	12 mg./lb.	14 mg./lb.	16 mg./lb.
5.0	2.27	0.67	0.83	1.00	1.16	1.33
5.5	2.50	0.73	0.92	1.10	1.28	1.47
6.0	2.72	0.80	1.00	1.20	1.40	1.60
6.5	2.95	0.87	1.08	1.30	1.52	1.73
7.0	3.18	0.93	1.16	1.40	1.63	1.87
7.5	3.40	1.00	1.25	1.50	1.75	2.00
8.0	3.63	1.07	1.33	1.60	1.86	2.13
8.5	3.86	1.13	1.42	1.70	1.98	2.27
9.0	4.08	1.20	1.50	1.80	2.10	2.40
9.5	4.31	1.27	1.58	1.90	2.22	2.53
10.0	4.54	1.33	1.66	2.00	2.33	2.67
10.5	4.77	1.40	1.75	2.10	2.45	2.80
11.0	5.00	1.47	1.83	2.20	2.57	2.93
11.5	5.23	1.53	1.92	2.30	2.69	3.07
12.0	5.45	1.60	2.00	2.40	2.80	3.20
12.5	5.68	1.67	2.08	2.50	2.92	3.33
13.0	5.91	1.73	2.16	2.60	3.03	3.47
13.5	6.14	1.80	2.25	2.70	3.15	3.60
14.0	6.36	1.87	2.33	2.80	3.26	3.73
14.5	6.59	1.93	2.42	2.90	3.38	3.87
15.0	6.82	2.00	2.50	3.00	3.50	4.00
15.5	7.05	2.07	2.58	3.10	3.62	4.13
16.0	7.27	2.13	2.66	3.20	3.73	4.27
16.5	7.50	2.20	2.75	3.30	3.85	4.40
17.0	7.73	2.27	2.83	3.40	3.96	4.53
17.5	7.95	2.33	2.92	3.50	4.08	4.67
18.0	8.18	2.40	3.00	3.60	4.20	4.80
18.5	8.41	2.47	3.08	3.70	4.32	4.93
19.0	8.64	2.53	3.16	3.80	4.43	5.07
19.5	8.86	2.60	3.24	3.90	4.55	5.20
20.0	9.09	2.67	3.33	4.00	4.66	5.33
20.5	9.32	2.73	3.42	4.10	4.78	5.47
21.0	9.55	2.80	3.50	4.20	4.90	5.60
21.5	9.77	2.87	3.58	4.30	5.02	5.73
22.0	10.00	2.93	3.66	4.40	5.13	5.87
22.5	10.23	3.00	3.75	4.50	5.25	6.00
23.0	10.45	3.07	3.83	4.60	5.36	6.13
23.5	10.68	3.13	3.92	4.70	5.48	6.27
24.0	10.91	3.20	4.00	4.80	5.60	6.40
24.5	11.14	3.27	4.08	4.90	5.72	6.53
25.0	11.36	3.33	4.17	5.00	5.83	6.67
25.5	11.59	3.40	4.25	5.10	5.95	6.80
26.0	11.82	3.47	4.33	5.20	6.07	6.93
26.5	12.05	3.53	4.41	5.30	6.18	7.07
27.0	12.27	3.60	4.50	5.40	6.30	7.20
27.5	12.50	3.67	4.58	5.50	6.42	7.33
28.0	12.73	3.73	4.67	5.60	6.53	7.47
28.5	12.95	3.80	4.75	5.70	6.65	7.60
29.0	13.18	3.87	4.83	5.80	6.77	7.73
29.5	13.41	3.93	4.92	5.90	6.88	7.87
30.0	13.64	4.00	5.00	6.00	7.00	8.00

* Total dosage given in milliliters.

† Sixty mg./lb.

weighing one-half to three-fourth lbs., approximately the size of a large orange (meal absorbs water equal to its weight).

Meal	50 lb.
Vi-Daylin	4 oz.
Ascorbic acid	5 gm.

This diet is prepared fresh each day and left-over food balls are discarded. Animals are fed once daily. Isonicotinic acid hydrazide (Isoniazid), 20 mg./kg.

FIGURE 3. Individual cage (developed at the Radiobiological Laboratory) used to house the main colony. Each cage is made of aluminum and has a small sliding door with snap fastener and feeding slot.

of monkey body weight, is also added to the above mixture as an aid in the prevention of tuberculosis. This is discussed in more detail below.

Each animal in the colony is weighed monthly. Individual and mean colony weights are maintained on graphs that provide a current index to the condition of the colony.

Preventive Medicine

There is no substitute for a good preventive-medicine program. In the research colony, a healthy normal monkey is expected by the primary investigator. It is imperative that the monkey remain healthy and live as long as possible. The following points of preventive medicine, which are followed rigorously, can be applied to almost any colony in any laboratory.

(1) Animals must pass three negative tuberculin tests during a 90-day quarantine period.

(2) Only necessary personnel are permitted in the animal quarters.

(3) Animal handlers must wash hands and equipment between handling groups of monkeys.

(4) Aseptic surgery is practiced in minor as well as major operations.

(5) A visual health appraisal is made of each caged animal both mornings and evenings. Loose stools are collected and examined for evidence of parasites and pathogenic bacteria.

(6) Every animal is weighed each month. Feeding problems, parasitic infections, and other colony problems often can be detected from the comparative body weights.

(7) Cages, dropping trays, and rooms are washed daily, and floors are mopped with 3 per cent Lysol solutions.

(8) Cages are steam-cleaned monthly, and carrying cages are cleaned and disinfected in a 3 per cent Lysol solution after each use.

(9) Animals suspected of being diseased are housed in isolation.

Disease Control

The monkey's reaction to disease is more acute than that of most animals. During the initial period he demonstrates great strength and ability to resist infections. Should the infection be overwhelming, a relatively normal-appearing animal may collapse and die within a few hours.

The significance of rectal temperature change in the monkey is difficult to evaluate. Clinical signs such as dehydration, condition of hair coat, and activity are more valuable aids in diagnosing diseases of the monkey.

Shigellosis and salmonellosis. The most common enteric bacterial diseases encountered are shigellosis and salmonellosis. After a colony is well established and proper sanitation is maintained, these bacterial outbreaks become infrequent. Transportation, overcrowding, receipts of new animals, and deficient or inadequate diets are factors that are conducive to disease outbreaks.

Shigellosis can usually be distinguished clinically from salmonellosis by the former's more acute course and severe diarrhea with marked hemorrhage. Both diseases, however, cause the general signs of gastrointestinal distress, dehydration, and weakness. Laboratory findings from stool cultures should be used to differentiate between shigellosis and salmonellosis. Newly acquired animals that have been exposed to infections are medicated prophylactically on a group basis. Noticeably ill animals are separated to individual cages and are individually medicated.

Tuberculosis. Preventing tuberculosis in the colony is imperative. A concerted effort is made to prevent all animals and human carriers from coming into contact with the colony.

An outbreak, reported by Benson *et al.*,[1] occurred during the winter of 1952–1953. During this epidemic, test and sacrifice practices were instituted even though many valuable animals were involved. The report "A Tuberculosis Outbreak in a *Macaca mulatta* Monkey Colony" by Benson and his colleagues treats this subject.

The following is an outline of the present colony antituberculosis program.

(1) The entire colony is routinely tuberculin-tested quarterly. Each monkey is caught and restrained at his cage. With the thumb on the supraorbital crest, the skin is pulled upward and 0.1 ml. (25 mg.) of undiluted B.A.I. Mammalian Tuberculin is injected intrapalpebrally.

(2) The test is read at 48- and 72-hour intervals. A plus (+) system of grading reactors is used. Under this system, one plus (+) is recorded if any erythema is observed; two plus (++) where erythema and edema are present; three plus (+++) if marked or extensive erythema and edema are manifested.

(3) All positive reactors, two- or three-plus (++ or +++) are promptly eliminated. A one-plus (+) reactor is usually considered the result of trauma and therefore considered to be a negative reaction. If tuberculosis is present in the colony, a one-plus (+) reactor may be regarded as a suspicious reactor, whereupon he is isolated and retested at 30 days. By this time a two- or three-plus (++ or +++) reaction will most likely be manifested if the animal has tuberculosis, due to the typically rapid course of tuberculosis in the monkey.

(4) Isoniazid, 20 mg./kg. body weight, is added to the diet as a prophylaxis to tuberculosis. This medication is reported (L. H. Schmidt, personal communication) to be effective in preventing the development and spread of tuberculosis in the monkey. This program has been in effect here for 3 years with apparently good results. Radiographs are considered of little diagnostic value for detecting tuberculosis. Findings have not been consistent nor as reliable as the intrapalpebral tuberculin tests.

Respiratory infections. Pulmonary infections at this laboratory have been infrequent; in other areas this disease is a major problem. Necropsies show signs of lobar consolidation and hemorrhage. The lungs are grossly consolidated, dark red, and "liverlike" in texture and appearance. Response to antibiotic therapy is usually undramatic. Infected animals that recover are weak and very susceptible to subsequent infections.

Ectoparasites. Ear mites have been found in some young monkeys. Fleas and lice are occasionally observed in newly procured animals. Diryl powder is applied for dry therapy and sulfur or chlordane, or both, for dipping. Canex and Lin-70 are used topically in the ears for mites.

Endoparasites. Each morning the individual feces are observed in the dropping pan. Any loose or abnormal stool is sampled and examined for parasites and streaked for bacterial culture. This procedure detects infections before they spread throughout the colony.

Two nematode species present a constant threat to good health. They are the *Oesophagostomum* sp. (nodular worm) and *Strongyloides* sp. *Oesophagostomum* produces nodular lesions in the wall of the colon. Phenothiazine is used to control this parasite. A heavily parasitized animal left untreated will become weak and may yield to secondary infections. Piperazine citrate (Parlamate) and phenothiazine have been used with varying results. Most favorable results have been obtained through using dithiazanine iodide, which may be included in the feed for colony treatment or may be given individually

at a dosage of 10 mg./lb. for 10 to 14 days, repeated if necessary until a final sampling is negative.

Tapeworm infections are rarely diagnosed. Tapeworm cysts have been found at necropsy, but these are mostly of academic interest.

Most imported monkeys harbor lung mites (*Pneumonysuss foxi*). Lung mites, common to the Indian-born rhesus monkeys, remain encysted in the lungs throughout the animal's life.[2] These cause little disturbance to the monkey, but their presence may confuse interpretations of pulmonary lesions. Two to three encysted mites per lung is a not uncommon autopsy finding. No attempt has been made to treat lung mites at this laboratory. Infants born here do not exhibit lung mites.

Amebiasis and balantidiasis. Endamoeba histolytica and *Balantidium coli* have been identified at this colony. Sporadic outbreaks of amebiasis often present a serious threat to colony health. Milibus and Aralen are effective when administered for 10 days. Carbarsone and Diodoquin are also effective amebiasides.

Weak monkeys. Occasionally a chilled and debilitated condition is observed, especially in the infant monkey. An apparently normal monkey will become acutely weak and prostrate. These animals will usually die in a few hours if left untreated. Treatment consists of providing external heat by placing the animal in an infant incubator (80 to 90° F.) and administering 10 to 20 cc. of warm 50 per cent dextrose via stomach tube. Pentylenetetrazol (Metrazol) or other stimulants are administered, and oxygen is supplied if respiration seems labored. A monkey treated in this manner usually responds within 2 hours. Normal solid food is fed as soon as the animal will accept it.

Supportive therapy. A Baby Haven infant incubator is used in both the treatment and surgery rooms. This equipment provides a holding area in which temperature, humidity, and oxygen can be controlled. This equipment is most useful in recovery of postsurgical cases, in infants that have become chilled and need this protection, in monkeys suffering from generalized infection that are under observation and therapy, and in those emergency cases demanding increased oxygen supply.

Supportive fluids are extremely important. Any disturbance in the electrolyte balance is immediately reflected by dehydration of eyes, skin, and hair coat. Five per cent dextrose and normal saline are administered at the rate of 100 ml./hr. Protein hydrolyzate solutions are indicated when the animal is off feed and requires additional protein. Whole blood is collected either in a syringe or vacuum bottle. A-C-D solution is used as an anticoagulant. Even though we have experienced limited blood groupings, these are not sufficiently strong to cause difficulty in transfusing the monkey. Five to ten cc. of blood per lb. body weight may be collected from the donor and administered at a similar ratio to the recipient. Plasma expanders are also used when blood is difficult to obtain.

Drug toxicity. Dihydrostreptomycin sulfate and streptomycin sulfate may cause toxic reactions when given at a dosage higher than 10 mg./lb. body weight. Symptoms follow 10 to 20 min. after injection, and the animal dies in convulsions.

Surgical and Incidental Procedures

Two common sites are used for bleeding and intravenous injection. The inguinal region is most frequently used. With practice, venous or arterial blood can be collected. The monkey is held on its back while blood is taken from the femoral artery or vein near the femoral triangle. A 21-gauge, 1½-inch needle works well when using the inguinal method. Injections can be made easily on the saphenous vein, but negative pressure will collapse this vein, making it almost entirely useless for exsanguination.

Passing an O. D. 0.048-gauge polyethylene tube through a 15-gauge needle into the saphenous vein has been an aid in obtaining good hematology specimens. Circulation timing, phlebotomizing, and random sampling are but a few of the uses for this method of collection.

Bone-marrow biopsies. The preferred site for representative bone-marrow biopsies is the ischial crest.

TABLE 4

Name	Common name	Habitat
Ateles sp.	Spider monkey	Central and South America
Cebus sp.	Capuchin	South America
Cercocebus-forquatus atys	Sooth mangabey	Africa
Cercopithecus-aethiops sabaeus	Green monkey (Gyenous)	Africa
Macaca irus	Cynomolgus	Asia
Macaca nemestrina	Pigtailed	Malay
Macaca philippinensis		Philippines
Macaca sinica		Ceylon
Saimira	Squirrel	Central and South America

Extraction of canine teeth. The animal is anesthetized with pentobarbital sodium (Nembutal) and an intratracheal tube is inserted. The pharynx is packed off with 4-by-4-in. gauze to prevent aspiration of blood. The gingiva is incised on the buccal surface one half of an inch, parallel to the long axis of the tooth. The bony plate is loosened by enlarging the socket with a root elevator. When the socket is sufficiently enlarged, an elevator is inserted in the space between the adjacent tooth and rotated so as to lift the tooth from the socket. Tooth forceps should not be used except to guide the tooth from the socket. Since the bicuspids have spiral roots, the force of extraction should be outward and away from the face. After extraction, all bony chips should be removed from the socket and the gums united by gut sutures.

Stomach tube. The stomach tube can be passed successfully through the mouth or nose. This procedure is simple and fast, when using a No. 16 red rubber catheter through the mouth.

Wound suture. Gut suture is commonly used for minor wounds. Wire sutures are satisfactory for severe-wound repair. Wound dressings other than Aeroplast or like material are virtually impossible to keep in place.

Blood pressure. Blood pressure is measured with an infant cuff on the upper

arm, using a sphygmomanometer. A range of 100–150/80–130 with a mean of 125/104 resulted from a group of 144 readings from 12 apparently normal monkeys. So that all of the animals' temperaments might be on a relative plane, the animals were tranquilized with Thorazine prior to each reading.

Rectal prolapse. Prolapsing of the rectum is not uncommon when large groups of monkeys are caught from colony cages. This condition will usually correct itself when the animal is left undisturbed. If it has not corrected itself after several hours, it should be manually reduced.

Necropsy. All dead animals are necropsied. Tissues are processed, evaluated, and filed for future reference.

Procurement

In the past, rhesus monkeys for this laboratory have been purchased from trappers of the Uttar Pradesh of India directly by Air Force personnel. The advantages of going to India as a source of supply include the following: (1) the source of natural environment is known; (2) a complete history of disease since captivity is obtainable; (3) a minimal duration of time is spent in transportation; (4) a minimal duration of time is spent in vendors' confinement and collecting stations; (5) tuberculosis reactors are eliminated at the source; and (6) the initial cost per animal is small.

Because of increased imports of *Macaca mulatta* monkeys into this country, it has become more economical to utilize the services of domestic importers. It is important to obtain the following information before contracting for animals: (1) the source of supply; (2) the name and reliability of the foreign trapper; and (3) the operative procedure and knowledge of the vendor's receiving and holding facility in the United States. Selection and tuberculin testing of new animals should be accomplished at the vendor's establishment.

Mortality in animals processed directly from India is estimated to be in the 20 to 30 per cent range. This may be attributed to lowered resistance as a result of chilling, overheating, and overcrowding during transportation.

Other monkeys. The rhesus (*Macaca mulatta*) monkey is the species most commonly used in research. Although there are more data in physiology and pathology for this species, other monkey species are being imported that may have certain advantages over the rhesus. Certain other species have the following advantages: (1) resistance to tuberculosis and other diseases; (2) the smaller size of the adults; and (3) greater ease of caging and maintenance.

Although they are not being used here, other laboratories have reported on one or more of the monkeys as experimental subjects (TABLE 4).

References

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CARE OF THE LARGE MONKEY COLONY

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The procedures and descriptions presented here illustrate the management of a receiving and holding colony from which large numbers of monkeys are withdrawn daily for vaccine production and for research purposes. There have been many excellent publications in recent years on the problems of simian primate care, primarily in the smaller investigational-type colony. This report will avoid repetition, but will of necessity emphasize certain husbandry practices previously presented[1-3] that are uniquely related to the large colony. This type of supply colony presents differences and problems that are minimized in the small unit, but that become of considerable economic significance when the colony grows in size. Probably the greatest basic difference between the two units is that a colony geared to a heavy production schedule does not reach a state of equilibrium. The monkeys submitted daily for kidney removal or test purposes are usually replaced each week by incoming shipments of the same or different species from widely separated geographical areas, whose members are in varying degrees of health. The potential for continuous reintroduction of pathogens is obviously greater than in the smaller colony that gains a low mortality figure sooner after the infrequent arrivals adjust to their new environment and have minimal or no contact with new monkeys.

Since large numbers of monkeys are transported principally by air, the problems to be considered begin when the animals land at the airport and end when the animals are submitted for kidney excision, to the vaccine test section, or to the research division.

Transportation of Monkeys from the Airport to the Colony

Trucks used for the transfer are positioned at the airport ahead of the plane's scheduled arrival time to avoid delay should the plane arrive early. A large tarpaulin is spread on the pavement between the plane and truck to catch grain and other feed that may be dropped. This procedure expedites cleaning up after the transfer. Since most shipments embark from a foreign country, the material collected is incinerated to curb introduction of agricultural pests.

The inside dimensions of the trailer used are approximately 34 feet in length, 7½ feet in width, and 7 feet in height. The vehicle is insulated with Fiberglas, and the interior is entirely lined for efficient disinfection with galvanealed steel with all joints welded. The unit will accommodate 150 standard Indian shipping crates 36 inches long, 21¼ inches wide, and 19 inches high. Heating in the winter and cooling in the summer are accomplished with an air-conditioning unit mounted outside on the front and powered by a separate 5-horsepower gasoline motor. Fresh air is admitted through 2 intake ports on the front and, after being mixed with recirculated air, passes through the conditioner. The air is then distributed through the trailer via a central ceiling duct running nearly full length with outlets along its sides. The rear of the trailer,

consisting of 2 large doors, contains 4 exhaust ports, 2 of which also serve as grilled inspection doors. A thermobulb is located inside the trailer, with the temperature gauge mounted outside in easy view of the driver.

The semitrailer is backed to the plane's cargo door for transfer and, in inclement weather, either a tarpaulin is tied between the trailer and plane, forming a covered aisleway through which the crates are passed, or a covered belt conveyer is used. The load is arranged with lengthwise aisles to provide for air movement through the trailer. Two recording thermometers are distributed within the load as maintenance checks on the air-conditioning equipment. The vehicle is stopped every half hour for inspection. After each use the trailer is swept clean, and refuse incinerated. The interior is scrubbed with an iodine-detergent disinfectant solution and rinsed clean with hot water. The disinfectant solution is applied again and allowed to remain for any continuing effect. The trailer is restricted to transporting monkeys.

The Monkey Quarters

The quarters are entirely inside and consist of room-type holding and conditioning cages. The large holding cages are in groups of 5 and open into a common aisleway. Floor dimensions of these cages are 14 feet by 8 feet, 8 inches. Conditioning cages are in groups of 8 facing onto one aisle; floor measurements are 6 feet by 3 feet, 8 inches. Cage and aisle height throughout the colony is approximately 6½ feet. Rooms comprising a single group of holding cages, or several groups of conditioning cages, will accommodate approximately 500 monkeys. Rooms are separated at least by floor-to-ceiling walls. The colony consists of several separate buildings containing from 1 to 3 rooms. Maximum cage capacity is rated at one 5-pound monkey per square foot of floor space. Under average inventory conditions 1½ or 2 square feet of floor space per animal are provided. Woven-wire, tile, and metal panels provide a continuous closure through which animals cannot escape and which prevent hiding.

Most cages have glazed structural-tile side and back walls with woven-wire cribbing panel (diamond mesh) across the top and front, and cage doors and runway aisles of the same material. All cage doors have key locks to preclude escapes, and all buildings are locked during off-hours to prevent and protect unauthorized visitors. In addition to the cage doors, there are at least 2 doors fitted with closers separating the animals from the outside.

Several 1¾-inch outside-diameter perch pipes run crosswise through each cage 18 inches from the top and, in addition, the large holding cages have a trapeze perch hanging on chains. One perch in each cage is 2¼ inches outside diameter and constitutes the overflow pipe for the continuous-flow drinking-fountain system that is fabricated of standard pipe fittings (FIGURE 1) and located only 4½ inches from the wire ceiling to prevent its being soiled by excrement. The drinking water is warmed to approximately 10° F. below room temperature by introducing a small amount of hot water via a mechanical mixing device. This makes the overflow pipe more comfortable for perching and tends to eliminate condensation that the animals love to lick and that is sure to be soiled with fecal material. A hardwood bench 7 inches wide and 24 inches from the floor is built along one side of each cage.

All cages have a cement floor on which is spread a 3-inch layer of white pine shavings (not sawdust). There is no wire floor over the shavings. Each large cage contains a floor drain, and the smaller cages have drains immediately outside the group. A 12-inch-high metal draft guard is fastened along the bottom of all wire cage fronts and doors, which also prevents monkeys from reaching out for spilled waste, food, or cleaning solutions when the aisle is being sanitized. The large cages have a 6-inch aisle curbing to prevent bedding, cleaning solutions, and water from flowing into the aisleway.

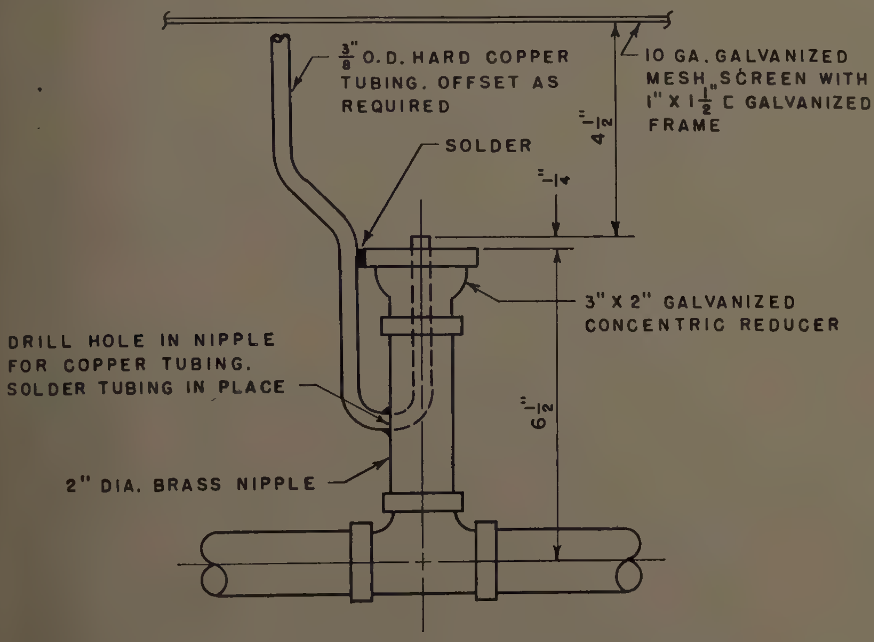

FIGURE 1. Monkey drinking fountain.

A night light is provided in each room so the animals are not in absolute darkness at night. These lights are 25-watt bulbs located overhead with a shield to reflect the direct rays to the wall. It is felt that dim light minimizes fighting and makes it easier for the animals to obtain food and water.

The buildings are air-conditioned the year around. The temperature, maintained at 78° F., is checked frequently during the day and recorded. Plant patrolmen check the temperature during off-hours, and service personnel are called when equipment failure is suspected, especially if the temperature drops below 74° F. or rises above 87° F. Air-inlet louvers are of a carefully selected design, adjusted to prevent drafts of air blowing directly down on the animals. The air is changed approximately 4½ times per hour, which is not great enough to cause chilling because of rapid evaporation or dryness. A slight positive pressure is maintained in each room.

Sanitation

Sanitation is maintained by completely cleaning and disinfecting each cage on the second day after it has been occupied. The animals do not occupy one cage continuously, but are herded through the aisle from dirty cages to clean cages on a continuous cycle with empty cages always available for cleaning purposes. Cages are cleaned by attendants who remain in and care for the animals in their assigned areas as much as is practicable. A record is maintained so that cages are cleaned in rotation and are not selected purely on the basis of appearance. If delays because of interruptions or absenteeism put the cleaning program behind schedule, the cages are cleaned on the third day at the latest, even if overtime work is required. The soiled litter is removed and incinerated, the cage is soaked and hosed down with hot water to remove the loose excrement and scrubbed clean with an iodine-detergent disinfectant solution. After this is rinsed off, a fresh application of the disinfectant solution is applied, allowed to remain in contact for 5 min., and completely rinsed off with plain water. The cage is then squeegeed dry and readied with shavings for receiving monkeys from the next cage to be cleaned, and the cycle is repeated. Steam is not used for cleaning, and fogging resulting from excessive use of hot water in an area occupied by monkeys is avoided. Feed pans are cleaned at the same time and in the same manner as the cages.

Movement of Monkeys

Movement of monkeys within the quarters is largely restricted to the cleaning operations described above. Animals from different incoming shipments are never mixed in the same cage and are kept in separate rooms if possible. Monkeys usually have been grouped according to size by the supplier. Once assigned to a cage, regrouping within the shipment is avoided as much as possible. The too-small animal, the too-large, the aggressive, the cage mates of tuberculin reactors and individuals not gaining weight are culled daily for kidney excision. The clinically ill are removed, treated, and sent for kidney excision when in overt good health, without being returned to their original cages. The best animals from each cage are removed and sent to the smaller conditioning cages. Grouping of original cage mates together is maintained as much as practicable as the monkeys progress to successively smaller cages, even to the vaccine test section. The inventory is arranged so that monkeys submitted for test or research purposes have been in the colony for a minimum of four weeks.

Feeding

Feeding is accomplished by using a mixture of two commercially prepared monkey feeds in extruded pellet and biscuit shapes. The mixture is basically wheat. The mixture is full-fed day and night in a loaf pan protected by a hooded shield that tends to retain feed scattered by the monkeys. The pan is hung on the aisle side of the wire cage front through which the animals reach for the food. Four pans are hung on each large holding cage and one pan on each conditioning cage. Each animal requires (consumption plus waste) an average of approximately one-third pound of feed per day using this system.

With the exception of bananas fed once per week, no other feed or supplement is given.

Medication and Aids to Diagnosis

Medication of all animals is conducted as soon as practicable after arrival as a prophylaxis against the enteric and pneumonic infections that appear so frequently in monkey colonies following receipt of new arrivals that have been stressed from transportation and other environmental changes.[4-6] One and one-half cc. of a mixture containing 200 mg. chloramphenicol (Chloromycetin*) and 400,000 U. of penicillin (S-R*) is given intramuscularly by automatic syringe in the anterior portion of the thigh. Every animal in the shipment is caught and injected for 3 consecutive days, or as practicable. If week ends or holidays interfere with this program, the first dose may be doubled and divided over 2 sites. A repeat dose is given once per week or as indicated for as long as the animals are maintained in the holding colony. Vermifuges or other drugs are not used routinely, and additives are not made to feed or drinking water.

Work is arranged so that the interchange of personnel and equipment between species and shipments or between the sick and well is minimized as much as practicable.

The tuberculin test is performed on new arrivals at the time of the first or second dose of antibiotics mentioned above. This is accomplished by injecting 7 to 10 mg. Old Tuberculin, U.S.P., intracutaneously in the right upper eyelid.[7,8] A positive reaction is characterized in 1 to 3 days by edema of the eyelid and surrounding tissue, accompanied frequently by lacrimation and redness. Reactors are euthanized and the carcasses incinerated. Tuberculin tests are repeated at approximately 2-week intervals or more frequently in cages where reactors have been found. The cage mates of reactors are submitted for kidney removal as soon as practicable.

In order to minimize the possibility of including an animal with pneumonia on test, monkeys submitted to the vaccine test section are fluoroscoped with an apparatus specially designed to eliminate radiation hazard to personnel (J. K. Weston *et al.*, to be published). Animals with significant lung pathology are withheld and treated as indicated. Monkeys are identified by tattooing the upper abdominal region.[9]

Animals are observed twice per day on a regular basis by technicians, and monkeys showing generalized clinical signs of illness are segregated. Medication is accomplished by injecting antibiotics for 3 consecutive days, as described above. In addition, Lactate Ringers Injection modified with 3 per cent dextrose is administered intraperitoneally at a dose of 22 cc./kg. body weight and modified fibrin hydrolyzate injection, low sodium, U.S.P. (Aminosol 5 per cent†) is given subcutaneously at a dose of 20 cc. per kilogram body weight. A liquid vitamin preparation (Abdec Drops‡) is administered by dropping 0.3 cc. on a cube of sugar. Brown, uncooked rice is offered in addition to the regular diet.

* Parke, Davis and Company, Detroit, Mich.
† Abbott Laboratories, North Chicago, Ill.
‡ Parke, Davis and Company, Detroit, Mich.

Safety Precautions

Certain precautions while handling monkeys are insisted upon to protect personnel from infections[10-12] apt to be carried by or infecting the simians. New employees are given a set of personal-hygiene instructions to read and are fitted with safety glasses before starting to work. They are also given immunizations against tetanus and smallpox and against other agents when advisable because of the close relationship between the supply colony and vaccine-

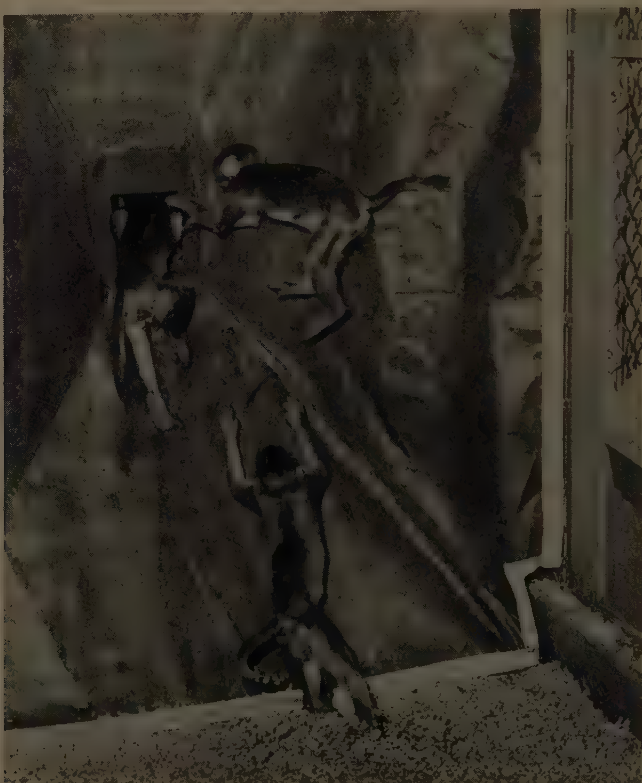

FIGURE 2. Monkeys are herded into a chute through an opening in an aisle curtain.

producing units. Chest X rays are taken every three months. Gauze face masks are worn at all times when in cage rooms. Gloves strong enough to protect against abrasions for the job at hand are required. Protective clothing worn when catching monkeys consists of: (1) eye protection, either safety glasses or goggles; (2) a parka hood on a long-sleeved jacket; and (3) thick leather gloves with gauntlets extended to elbows. Work clothing is autoclaved after use and before being sent to the laundry. Soiled clothing and footwear are changed before meals and before going home.

The problem of frequent catching of many hundreds of monkeys for medication has been facilitated with the aid of a trapping device, which eliminates chasing and tends to reduce the number of bites and scratches to personnel.

The trap is used in conjunction with a curtain (FIGURE 2) stretched across the aisleway, and which contains a central opening through which the monkeys are herded into a chute (K. Young; personal communication, 1955). Once in the chute (FIGURE 3), a top-hinged, transparent Plexiglas door is dropped behind the monkey and he is advanced to the end of the chute, if he is not already there, by means of a rolling carriage to which the hinged door is attached.

FIGURE 3. Monkeys are trapped at the end of a chute and removed through a sliding door on top. The animals are advanced to the trap end by a rolling carriage bearing a one-way top-hinged Plexiglas door.

This confines the animal for simple removal through a sliding top door. This device can also be adapted to run monkeys into carrying cases or other chambers. The curtain is made of 16-oz. nylon neoprene coated on both sides. It has sail slides fastened along the sides that slide on sail track mounted permanently on the aisle walls. The top and bottom are held taut with metal rods in hem pockets.

Observations

With the physical construction, animal husbandry, and medication program described above, this colony has experienced a mortality of 0.137 per cent per

day or 4.163 per cent per month for a 1-year period, October 1958 through September 1959. These are the latest available figures and were derived from the average population or inventory and average mortality. Records covering the first 24 hours after receipt were not used.

Computing mortality on the basis of total monkeys received and those dying after 24 hours until the animals are used completely, the colony experienced a mortality of 3.03 per cent from August 1958 through July 1959, which are the latest available records. "Completely used" in this instance refers to that point at which the animal has been submitted for kidney excision, placed on test, or the few remaining assigned elsewhere to long-term studies.

The fluoroscopic examination has revealed an incidence of 1.78 per cent pneumonia or 38 cases in 2,138 rhesus fluoroscoped from March through August 1959. This compares with an incidence of 20.92 per cent or 439 cases in 2,098 rhesus fluoroscoped from March through August 1955. In both instances the monkeys were considered in overt good health and were screened for test or research purposes. In the 1955 group the monkeys had been in the colony for an average of 19 days, whereas in the 1959 group they had been in the colony an average of 45 days since receipt. During the earlier period many of the practices described above had not as yet been instituted.

Acknowledgments

We acknowledge the assistance of the engineering staff of Parke, Davis and Company for their ideas and cooperation in constructing quarters and equipment, especially John C. Niemi for his work on the air-conditioned trailer, Richard R. Melcher for his work on the construction design, and C. M. Tyler for his work on the monkey trap.

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MASS TREATMENT OF MONKEYS WITH ANTIBACTERIAL SUBSTANCES

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Introduction

In any animal colony, the prevention and control of infectious disease is a problem of paramount importance, and this is true especially in large dynamic colonies of monkeys where the periodic arrival of new animals provides a constant source of potential infection and reinfection.

Studies have revealed that the majority of monkey deaths are due to infections of the respiratory and digestive systems, although stress and malnutrition usually are contributory factors (Sauer and Fegley, this monograph).

In a series of 240 autopsies performed on monkeys that died during the first 7 months of 1959, 37 per cent had pneumonia, 27 per cent had enteric infection, and 24 per cent had both pneumonia and enteric infection.

In addition, autopsies of apparently healthy monkeys revealed that a high percentage of the monkeys held in the colony less than 1 week had gross lesions of pneumonia. For example, autopsies performed on 123 apparently healthy cynomolgus monkeys from 8 different shipments revealed that 63 per cent had gross lesions of pneumonia and 52 per cent of 45 rhesus animals had similar lesions. Pneumococci streptococci, and micrococci were the pathogens most frequently isolated from the respiratory tract.

In the rhesus monkey, enteric infection accounted for more losses than any other single factor. This has been reported also by Habermann and Williams,[1] Hardy,[2] and Ruch.[3] The infection usually existed in the group at the time of arrival. Many of the animals had diarrhea that was watery, catarrhal, or hemorrhagic. In 3 large rhesus shipments a total of 86 autopsies was performed on monkeys either dead on arrival or dead within 24 hours after arrival. Seventy-one per cent had obvious enteric lesions. *Shigella flexneri* appeared to be the agent responsible for enteric infection. Further information regarding the bacteriological findings in the colony has been reported elsewhere (Sauer and Fegley, this monograph, Prier *et al.*[8]).

In our experience, cynomolgus monkeys usually do not have enteric infection at the time of arrival.

Numerous investigators have reported on chemotherapy in individual monkeys or in groups of monkeys.[2-7] In this study, mass treatment is emphasized, and comparisons are made of the losses in groups receiving treatment and in untreated control groups. Mass treatment involves the use of therapeutic agents on all animals within a group regardless of the number having clinically apparent disease. The choice of drugs was based upon effectiveness and ease of administration. When dealing with large numbers of animals, parenteral

administration consumes much time and labor and predisposes to injury of both animal and man. As diseased monkeys will not eat but usually will drink, products that could be administered in the drinking water were used where possible.

The primary objective was to find chemotherapeutic substances that would fulfill these requirements; however, the drugs finally chosen did not represent the only products available for this purpose.

Clinical trials were arranged to evaluate the efficacy of chemotherapeutic agents under various circumstances. These trials and the results obtained will be discussed.

Materials and Methods

Animals and Quarters

The colony was composed of approximately 1800 *Maca mulatta* (rhesus) and *Macaca philippinensis* (cynomolgus) monkeys. The proportion of each varied, but approximately two thirds of the colony was rhesus and one third was cynomolgus. The animal quarters were divided into separate units. Each unit contained from 3 to 8 chain-link gang cages, each having a capacity of from 100 to 200 animals per cage. The cages were approximately 7 feet high, 15 feet wide, and 20 feet long.

Antibacterial agents

Dosages of water-soluble substances were calculated on the basis of an average consumption of 8 gal. of fluid per 100 monkeys in a 24-hour period. The antibiotics and chemicals chosen for trial were as follows:

Tetracycline (*Polyotic*). Thirty mg./lb. of body weight per day was given by adding 115 gm. of Polyotic to each 4 gal. of water.

Neomycin. The dose was approximately 67 mg./lb. of body weight per day. This was given by adding 20 gm. of 67 per cent neomycin powder for each 4 gal. of water.

Nitrofurazone (*Furacin Water Mix*). The dose was 165 mg./lb. of body weight of 4.59 per cent nitrofurazone, equivalent to 7.5 mg./lb. per day of pure nitrofurazone. This was given by adding 33 gm. of Furacin Water Mix to 4 gal. of water.

Oxytetracycline (*Terramycin*). Twenty mg./lb. of body weight per day was administered intramuscularly.

Furazolidone (*Furoxone Aerodust*). The dosage level was 0.02 per cent concentration in the feed, prepared by adding 20 gm. of Furoxone Aerodust to 50 lb. of feed.

Streptomycin-bacitracin (*Entromycin*). Based on feeding 100 monkeys 50 lb. of feed daily, the dose was approximately 25 mg./lb. of body weight per day of streptomycin and approximately 250 U. of bacitracin per lb. of body weight daily. This was prepared by adding 1 lb. of Entromycin to each 50 lb. of feed, but due to the waste encountered in feeding monkeys the amount actually consumed was probably at least one-third below this level.

Methods of Administration

Tetracycline, neomycin, and nitrofurazone are water-soluble and were administered to the monkeys in their drinking water. Furazolidone (Furoxone Aerodust), also water-soluble, was dissolved in the water used to wet the monkey mash. Streptomycin-bacitracin (Entromycin) is not water-soluble, but a suspension of this drug in water was used to wet the monkey mash. Oxytetracycline was injected intramuscularly.

Duration of Treatment

When used therapeutically, the drugs were given for a period of 4 to 6 consecutive days. When used prophylactically, they were administered continuously over a period of 2 to 4 weeks.

Results

The Use of Antibacterial Substances in the Treatment of Enteric Infection

A shipment of approximately 1400 rhesus monkeys was received at the colony. Seventeen animals were dead on arrival (DOA) and an additional 12 monkeys died within the following 24 hours. At post-mortem examination, 18 showed inflammation of the colon and 11 had pneumonia.

At the time of arrival, the entire group was given tetracycline in the drinking water. Although there were 12 deaths within 24 hours after arrival, there were only 18 deaths during the following 12 days.

Tetracycline appeared to control both the pneumonia and enteric infection initially. After two weeks, numerous cases of hemorrhagic colitis were again noted, even though the monkeys still were receiving tetracycline in their drinking water.

Several possible explanations were considered for the recurrence of the hemorrhagic colitis, including the possibility that the continued use of the antibiotic may have interfered with the normal intestinal flora, resulting in diarrhea. In order to determine the value of tetracycline and other antibacterial agents in controlling this recurrence, 5 cages of 100 rhesus monkeys each were treated and 2 cages of 100 rhesus monkeys each were retained as untreated controls.

In TABLE 1 it may be seen that there were 27 deaths in the untreated group in a 3-day period. This represents 13.5 per cent of the 200 untreated monkeys. In the 5 treated cages there were 15 deaths, or 3.0 per cent of the 500 treated monkeys. In other words, approximately 4 times as many deaths occurred per cage in the untreated group.

The combination of tetracycline in water and streptomycin-bacitracin combination in the feed appeared to be most effective in controlling enteric infection (TABLE 1). Nitrofurazone also appeared quite effective, but in other trials it proved much less effective. As cage No. 2 (which had been on continuous tetracycline therapy for over 1 month) had 8 deaths, it appears that tetracycline was no longer effective against the offending organism.

In order to evaluate the efficacy of treating a group immediately upon arrival at the colony, 6 groups of 175 each were placed in each of 6 gang cages within one unit. There were 52 DOAs and 24 deaths within 24 hours. Post-mortem

examination on 25 of these animals revealed gross lesions of colitis in 22. Ten of the 25 had pneumonia.

Immediately after unloading and caging, antibiotics were given to 3 groups, and 3 groups remained untreated. In TABLE 2 it may be seen that 12.4 per

TABLE 1

EFFECT OF THERAPY WITH ANTIBACTERIAL SUBSTANCES IN FEED AND WATER

Group No.*	Therapy	Total deaths for 3-day period
1	Tetracycline in water Streptomycin-bacitracin in feed	2
2	Tetracycline in water	8
3	Nitrofurazone in water	2
6	Tetracycline in water Streptomycin-bacitracin in feed	0
7	Streptomycin-bacitracin in water Streptomycin-bacitracin in feed	3
	Total	15
4	No treatment	17
5	No treatment	10
	Total	27

* One hundred monkeys per group.

TABLE 2

EFFECT OF THERAPY WITH ANTIBACTERIAL SUBSTANCES IN FEED AND WATER

Cage No.*	Therapy	Total deaths for 3-day period
1	Tetracycline & neomycin in water	2
2	Tetracycline & neomycin in water	3
3	Tetracycline in water, streptomycin-bacitracin in feed	6
	Total	11
4	No treatment	11
5	No treatment	28
6	No treatment	26
	Total	65

* One hundred and seventy-five monkeys per cage.

cent (65) of the 525 untreated monkeys died in this 3-day period as compared to 2.1 per cent (11) of the 525 treated animals. Six times as many deaths occurred in the untreated group.

As shown in TABLE 2, tetracycline and neomycin combined in the drinking

water appeared more effective than the combination of tetracycline in the water and streptomycin-bacitracin in the feed.

The Use of Antibacterial Substances in the Prevention of Enteric Infection

Recurrence of enteric infection in rhesus monkeys during the first 4 to 6 weeks after arrival was so frequent that it was decided to incorporate medication in the feed during the first few weeks in an atttmpt to prevent these outbreaks.

Our clinical impressions and a review of literature[5,9] indicated that furazolidone and streptomycin-bacitracin may have value in preventing these recurrences. It was found that prophylactic therapy utilizing these drugs did not eliminate all recurrences.

In an effort to strengthen the clinical observation that prophylactic therapy with furazolidone and streptomycin-bacitracin was of value, 540 rhesus monkeys were divided on arrival into 3 equal groups and were caged in a unit that was isolated from all other monkeys. All 3 groups of monkeys were treated for 6 days with tetracycline and neomycin. After the first 6 days, the first group of 180 monkeys was given a 0.02 per cent concentration of furazolidone in the feed. The second group of 180 monkeys was given streptomycin-bacitracin. The third group of 180 monkeys received no further medication and served as a control.

Recurrence of enteric infection first became apparent in the control group 7 days following the cessation of treatment with tetracycline and neomycin. On the eighth day there was 1 death in the control group and on the ninth day there was a second death. Both monkeys had gross lesions of colitis. At this time, an additional 13 monkeys in the control group had diarrhea. Approximately one third of these had blood present in the stool. Because of the recurrence of enteric infection, the control group was given tetracycline and neomycin again in the drinking water.

Eight days after this outbreak in the control group, or 16 days after cessation of treatment with tetracycline and neomycin, enteric infection was also noted in the group on streptomycin-bacitracin combination. Two deaths occurred in this second group, and an additional 8 were noted that had diarrhea. This group also was given tetracycline and neomycin again at this time to control the infection.

In this particular instance, the monkeys in the furazolidone group did not have any clinical evidence of recurrence of infection during the first 4 weeks following arrival. On the basis of these results and past clinical experience, it is believed that streptomycin-bacitracin combination or furazolidone (particularly the latter) can be used to advantage in aiding in the control of enteric outbreaks.

Combined Use of Therapy and Prophylaxis to Control Enteric Infection

The therapeutic combination of tetracycline and neomycin followed by prophylactic furazolidone therapy was more effective than other treatment regimes employed for enteric infection. This is well illustrated by a comparison of the 3 large rhesus groups shown in FIGURE 1. Group 46 received this treatment re-

gime with the exception of a few instances where streptomycin-bacitracin combination was substituted for neomycin.

All animals in groups 28 and 39 were treated with tetracycline upon arrival and several other drugs during the following 3 months, but neither neomycin nor furazolidone was employed. Nine losses occurred in group 28 and 11 deaths in group 39 during the first week. More losses were expected in group 46 than in either groups 28 or 39 because there were so many monkeys that were ill and dying at the time of arrival. Also, it is noted (TABLE 2) that one half of the monkeys in group 46 were not treated initially. Of 82 deaths during the first week, the majority (57) occurred in the 3 cages that were initially untreated. However, the losses for the ensuing 3-month period were only 2.6 per cent, as opposed to 10 per cent for group 28 and 7.9 per cent for group 39.

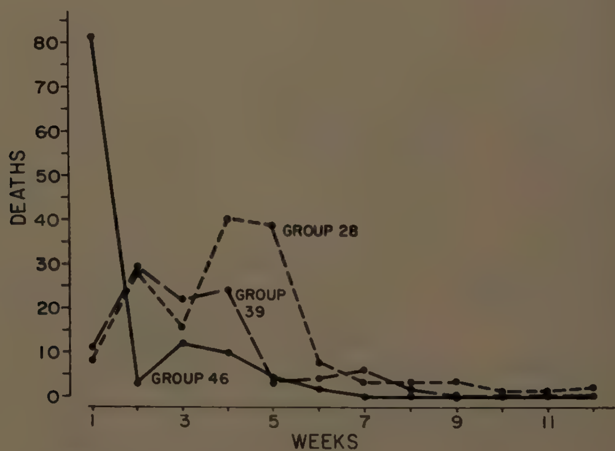

FIGURE 1. Comparison of losses in 3 large rhesus shipments (1230 to 1450 on arrival) that received varied treatment regimes.

The therapy employed in group 46 was much more effective than the treatment given to groups 28 and 39.

The Use of Antibacterial Substances in the Treatment of Respiratory Infection

In January 1958 there were many monkey deaths due to respiratory infection. An attempt was made to determine the value of antibiotics in the control of respiratory infection.

The animals involved were a group of approximately 1300 cynomolgus monkeys. There were 67 deaths on arrival. The monkeys, upon arrival, were placed in 6 gang cages with approximately 225 monkeys in each cage. There were 86 deaths in 24 hours and 256 dead in the following 7 days. Because of these losses, approximately 175 monkeys remained in each cage at the time the experiment was initiated. Two cages of monkeys were injected with oxytetracycline (20 mg./lb. I.M.) daily for 3 days. Two similar groups of untreated animals were held as controls. In the 7 days following the first injection of oxytetracycline, there was a total of 12 deaths in the 350 treated

monkeys and 61 deaths in the 350 untreated monkeys. This means that 5 times as many deaths occurred in the untreated control animals.

The monkeys in the 2 remaining cages also received oxytetracycline (20 mg./lb. I.M.) daily for 3 days. There was an average of 19 deaths per day during 3 days preceding treatment, whereas in the week following the second injection of oxytetracycline there was an average loss of 3⅓ monkeys per day.

Based on clinical observation, it appears that tetracycline administered in the drinking water is more effective than oxytetracycline administered parenterally. The administration of medication in the drinking water instead of by injection has many other obvious advantages: (1) less personnel is required to carry out the treating schedule; (2) it is not necessary to handle the monkeys, which eliminates the chance of injury to both men and monkeys; and (3) the elimination of handling reduces the stress upon the monkeys.

Discussion

As noted in the results, mass antibacterial therapy reduced losses significantly. The losses within the entire colony over an extended period of time were much higher prior to extensive antibacterial therapy. From October 1957 to May 1958, 43.3 per cent of the monthly inventory died.* During the period from October 1958 to May 1959, when extensive mass therapy was employed, 6.6 per cent of the average monthly inventory* was lost. The average inventory for both periods was the same.

Based on the results of the experiments, the following regime appears most advisable.

(1) As cynomolgus monkeys received in the colony usually do not have enteric infection but have a high incidence of pneumonia, tetracycline is given for 4 to 6 days to all cynomolgus groups at the time of arrival.

(2) As rhesus monkeys usually have a high incidence of enteric infection and frequently pneumonia as well, tetracycline with neomycin is given to all rhesus groups at the time of arrival. After the initial treatment schedule is completed, 4 to 6 days after arrival the entire rhesus group is given furazolidone as a prophylactic measure to aid in preventing enteric outbreaks.

(3) If, at any time after the initial 4 to 6 days on antibiotics, there are indications that an infection is present and spreading in any group of monkeys, this group is again given therapeutic doses of antibacterial agents. If the infection is respiratory, the group is given tetracycline. If the infection is enteric, the group is given tetracycline with neomycin for 4 to 6 days and then given prophylactic doses of furazolidone or streptomycin-bacitracin combination.

(4) When possible, cultures should be taken to identify the pathogenic organisms, and sensitivity tests should be performed to support the choice of the antibiotic or chemotherapeutic agent being employed.

Summary

In an effort to control enteric and respiratory infection and thereby reduce mortality in a large dynamic monkey colony, mass antibacterial therapy was employed.

* Exclusive of DOA and deaths within 24 hours.

Concomitantly with the employment of mass therapy, a study based upon autopsies and culture was undertaken to determine the causes of infection and the associated pathology. Autopsies of monkeys revealed that there was a high incidence of enteric infection and pneumonia. Cultures from the large intestines and the lungs indicated that bacterial pathogens probably were the etiological agents of the enteric and respiratory pathology noted.

Four experiments were designed to determine the effect of therapy on treated groups as compared to monkeys not receiving treatment. In this study it was noted that the losses in the untreated groups of monkeys were 4 to 6 times as great as in the groups given medication. The losses in the colony in an 8-month period prior to extensive antibacterial therapy were 5 to 6 times as great as in the same 8-month period the following year when mass therapy was employed.

Of the chemotherapeutic agents used in these studies, it was found that tetracycline administered in the drinking water was effective in controlling uncomplicated respiratory infection, and a combination of tetracycline and neomycin given in the drinking water was effective in controlling enteric infection. Furazolidone incorporated in the feed appeared effective as a prophylactic measure against recurrence of enteric outbreaks.

Mass antibacterial therapy proved practicable and effective in controlling enteric and respiratory infections.

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STUDIES OF THE EFFECTS OF BRAIN LESIONS ON SOCIAL BEHAVIOR IN *MACACA MULATTA*: METHODOLOGICAL AND THEORETICAL CONSIDERATIONS

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This paper will describe some of the techniques developed for the systematic study of social behavior in the monkey and will illustrate their use in investigating the effects of one class of physiological variables, that of brain lesions.

Those variables that influence the social behavior of *Macaca mulatta* may have relevance to the study of human pathological alterations in social or interpersonal behavior in terms of neuroses and psychoses as defined by Sullivan.[1]

Group Cage Behavior

Prior to performing brain surgery on the monkeys, control or base-line levels of social behavior are determined. The following technique is used. Groups of from two to eight monkeys are housed in large cages. Each day, after twenty-four hours of food deprivation, they are observed in a competitive feeding test during which the investigator drops about fifty food pellets, one at a time, into the cage through a feeding pipe and records the aggressive-submissive behavior that occurs. FIGURE 1 shows five animals sitting near a feeding pipe in the course of a testing session.

The aggressive behavior observed includes biting, chasing, and threat gestures. Submissive behavior is exemplified by cringing and running away from another animal. In addition to recording these interactions, the investigator maintains a record of which animal in a group obtains each pellet of food. A typical record sheet is shown in FIGURE 2. The legend provides an explanation of the use of the scoring form.

After several monkeys have been together for a short while the aggressive interaction becomes unidirectional. Thus if animal A attacks animal B, B almost never retaliates against A; instead, B will chase or bite C or D. It is also true that if A attacks B and B attacks C, then A may attack C with impunity. Such a social system is termed a "straight-line" dominance hierarchy. In referring to the position occupied by an animal within this miniature social system we may speak of its dominance "rank" in the hierarchy. Thus the animal with rank 1 is that member of the group that may attack any other member of the group. Similarly, the animal with rank 2 may attack any group member except the animal with rank 1. It does not necessarily follow that an animal's dominance rank will correlate highly with the number of bits of food it obtains or with the numbers of aggressive actions it takes; however, intercorrelations among these three measures are invariably high.[2] The animal with rank 1 will obtain at least 80 per cent of the food dropped into the cage in the competitive-feeding test situation and will also tend to engage in more aggressive behavior than any other animal. The quantitative meas-

ures of number of aggressive acts and number of bits of food are used to provide more sensitive indices of change than would be supplied by the ordinal dominance ranks.

Some examples of the typical result obtained with 2 groups of monkeys are illustrated in FIGURE 3. For each group the scores graphed are the total dominance scores for each member of a 5-monkey social group. The total dominance score is the sum of all the interanimal aggressive-behavior tallies and the number of instances of food-getting behavior during a day's session. The points in each graph represent the average of a 5- or 10-day period. In the studies from which these results were taken,[3] there was no significant effect of

FIGURE 1. Five macaques congregated about the feeding pipe during a competitive-feeding test.

the experimental treatment (surgery) on the most dominant animal, so that these figures demonstrate the stability and regularity of the patterns of interaction seen in monkey groups. Such stability had been observed for periods of 7 months or longer.[2]

Individual Cage Behavior

In addition to being observed in groups, the animals are observed alone in individual cages. Ratings are made of each animal's fearlessness in approaching the observer for food. There is considerable variation among animals; one animal will vocalize loudly, sit up at the front of its cage and, without moving from there, will accept as many pieces of food as the experimenter will offer. Another animal will cower silently at the back of the cage, cringe at the sight of the human observer, and refuse to accept food from him. FIGURE 4 is the form on which several types of behavior are recorded; the legend provides

an explanation of the categories of responses of the animal. These measures of behavior also exhibit considerable stability;[3] as in the case of the group-cage rating, they are used to provide base-line measures against which to evaluate the effects of an experimental treatment.

Application of Techniques

The data obtained with these techniques indicate that monkey social behavior is orderly and stable and that each animal has a characteristic pattern of behavior in response to both the human observer and other members of its own group.

Selected members of the groups have had portions of their brains removed or destroyed in order to study the effects of such lesions on social behavior.

Lesions within three structures have been studied; these structures are the amygdaloid complex,[4] the hippocampus (A. F. Mirsky and H. E. Rosvold, unpublished observations), and the cingulate gyrus.[3] They were selected for study because they form part of a phylogenetically old region of the brain, the limbic system that, as Papez[5] and MacLean[6] have suggested, constitutes a system for the expression and maintenance of emotional behavior. Of particular relevance to this hypothesis and to the studies to be described is an experiment performed by Klüver and Bucy.[7] These workers removed bilaterally the temporal lobes of a number of macaques and produced an animal that displayed "profound changes in emotional behavior . . . forms of motor and vocal behavior as are associated with anger and fear are not exhibited . . . all expressions of emotions . . . may be completely lost"[7] (p. 152).

This removal of the temporal lobes interferes extensively with the integrity of the limbic system in that the amygdala, the hippocampus, and the adjacent medial cortical tissue are removed.

Many of the changes in behavior described by Klüver and Bucy as changes in "fear and anger" were inferred from the fact that the operated animal's response to other monkeys and to the human observer was changed. It was on the basis of such information that these authors concluded that "there may even be a complete loss of emotional responses." Since the change was most manifest in the animal's social behavior, it seems that the above is a most appropriate type of behavior to study.

Examples of the results obtained with the three different lesions are shown in FIGURE 5. Each lesion has the effect of decreasing fear in the animal toward the human observer; thus all individual-cage animals achieve higher fearlessness ratings after surgery. On the other hand, only the amygdala lesion has any effect on the group-cage dominance scores; paradoxically, the effect is to reduce sharply the dominance scores of the animals with amygdala lesions and to reduce their ranks in the hierarchy as well. Although the contrast between the change in the two test situations is puzzling, the fact that lesions in the amygdala may produce unique effects is not surprising. Pribram and Kruger,[8] reviewing evidence from a variety of neurological disciplines, have concluded that the amygdala is part of two subsystems within the limbic system (the "first" and "second" systems) that are distinct from that subsystem (the "third" system) which includes the hippocampus and the cingulate gyrus.

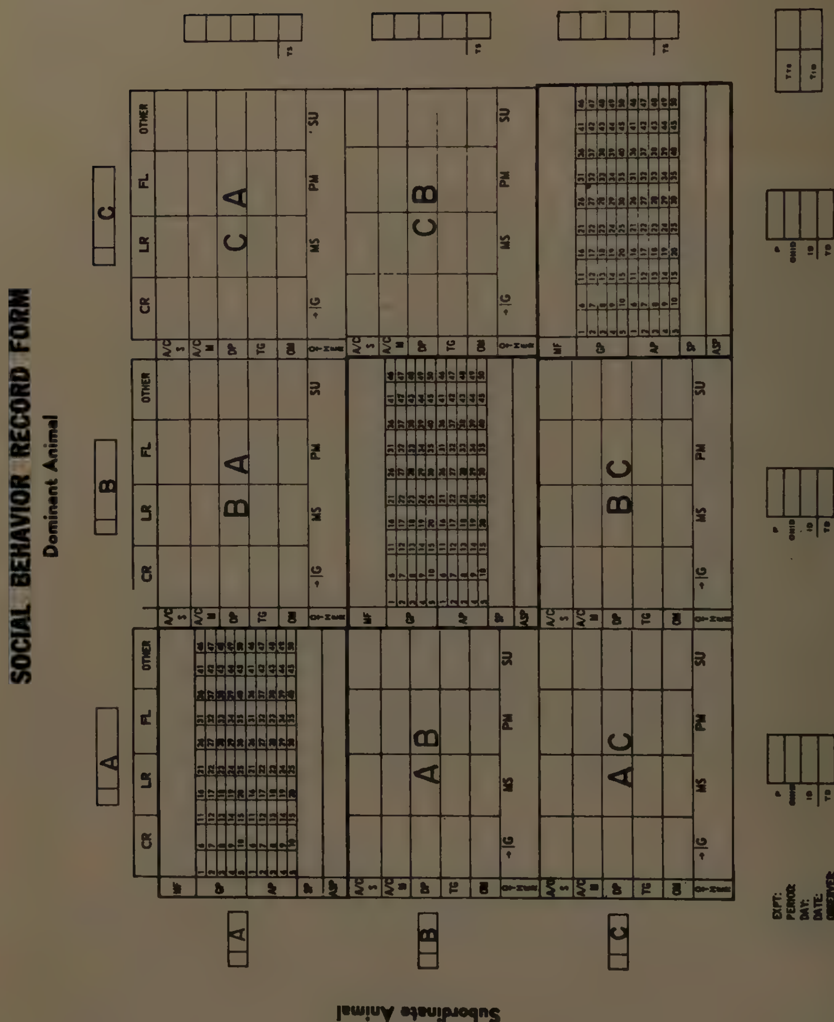

SOCIAL BEHAVIOR RECORD FORM
Dominant Animal
Subordinate Animal
EXPT:
PERIOD:
DAY:
DATE:
OBSERVER:

FIGURE 2.

FIGURE 6 summarizes the results obtained with twenty-two operated animals studied in these experiments.

How are these results and, in particular, the apparently opposite effects of the amygdala lesion in the two test situations, to be interpreted? An interpretation that has been tentatively adopted is that all these lesions have the effect of destroying or weakening previously learned connections between complex social stimuli and socioemotional responses. The loss or forgetting of learned responses to the human being is manifested in the following way: the operated animal responds directly and simply to the offer of food by accepting it readily. This would account for the apparent increase in fearlessness in the individual-cage situation. The amygdala lesion produces more of such impairment or "forgetting" than do the other limbic lesions, and this is reflected in the group-cage situation. The animal's cage mates, which are unimpaired, seem to sense its uncertainty and hesitation and, according to their own patterns of social response (their own aggressiveness), may attack and then dominate a previously dominant animal. Since the "retraining" afforded the operated animal by its cage mates in the immediate postoperative period is usually in the nature of aggressive attacking, it "learns" to be subordinate and passive.

A partial test of this critical postoperative relearning hypothesis has been attempted (A. F. Mirsky and H. E. Rosvold, unpublished observations). If it is true that the amygdalectomized animal must "relearn" social behavior, then it should be possible to "train" it to be even more dominant postoperatively than it was preoperatively (the opposite of the typical result) by an appropriate manipulation of its postoperative experience. The results of a preliminary experiment designed to test this prediction appear to offer support for the hypothesis. Three amygdalectomized monkeys were placed with groups of small young animals that they could easily dominate. When returned later to the groups to which they had originally belonged one of these animals *rose* one rank in the hierarchy and remained there, one rose temporarily, and all showed a significant if temporary increase in the number of instances of aggressive behavior shown toward the animals below them in the hierarchy. In no instance did any fall in rank. Some of the results of this study are illustrated in FIGURE 7.

These results provide some tentative support for the hypothesis that amygdala lesions impair previously learned, complex socioemotional stimulus-response connections. Other investigators of limbic-system function have advanced similar hypotheses. Weiskrantz and Wilson[9] and Thomas and Otis,[10] specifically, have also invoked learning, rather than exclusively emotional mech-

←FIGURE 2. The record form used for the scoring of social behavior in a single session in a group of three animals. Instances of food-getting behavior are scored separately for each animal, along the diagonal. Instances of dominant-submissive interaction are scored in the remaining boxes, two being provided for each pair of animals. Thus box AB is used to score an act in which A was the aggressor against B, and box BA for the reverse. Within each box are spaces for scoring all the commonly occurring combinations of dominant and submissive acts. In this way, a sequence such as "after peanut 5 is dropped, animal A bites animal C and animal C flees to the opposite end of the cage" may be scored simply by placing a number 5 in the AC box within the space provided for the appropriate dominant-submissive combination. A description of many of the behaviors scored with this technique has been published.[2] Additional details concerning the scoring system and the abbreviations used are available.

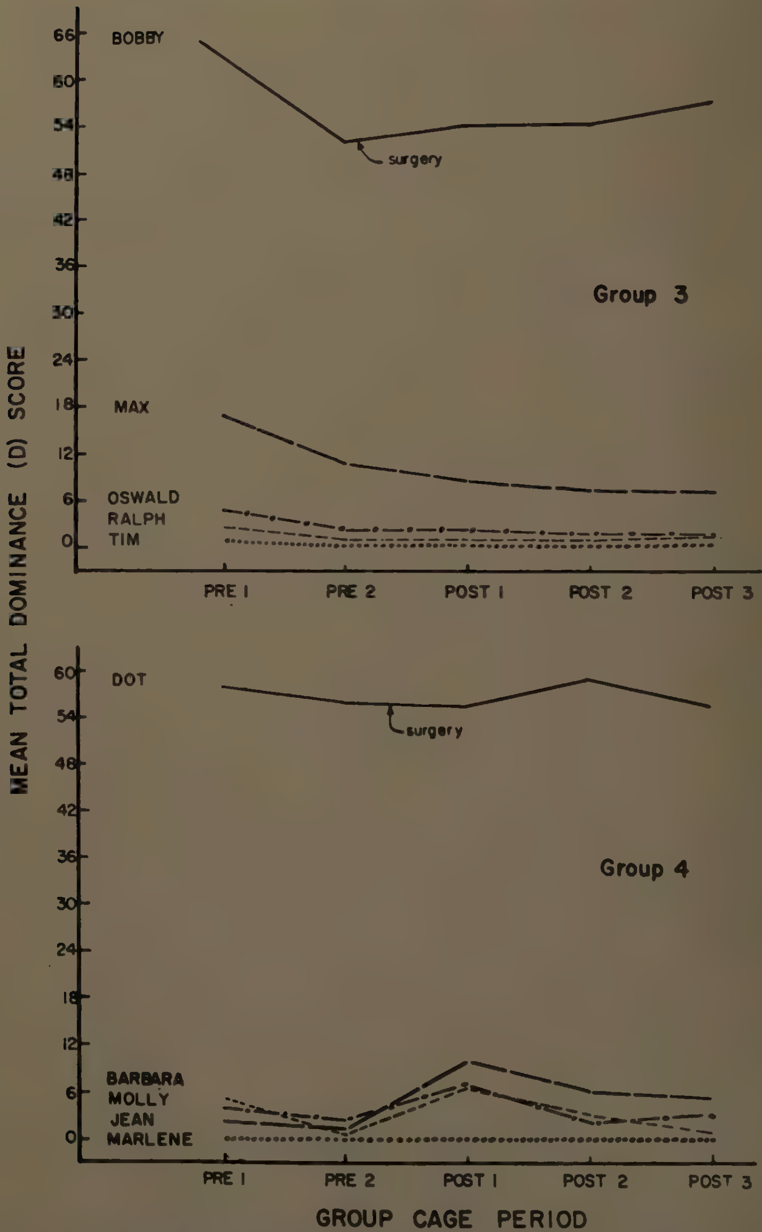


FIGURE 3. These graphs, made from the study of two different groups of five animals, illustrate some typical results obtained with the social-behavior scoring system described in the text and the stability of the measures of social behavior over time. Reproduced by permission of the *Journal of Neurophysiology*.[3]

anisms in accounting for the effects of limbic-system lesions. For example, Weiskrantz[11] has noted that "... the effect of amygdalectomy ... is to make it difficult for reinforcing stimuli, whether positive or negative, to become established or to be recognized as such ... immediate postoperative experience

Individual Cage Behavior Scale

Experiment:

Period:

Days: 1= 2= 3= 4= 5=

Notes: 1- 2- 3- 4- 5-

ANIMALS

Category	Rating	Score	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Vocaliz.	N n s	+2 +1 0																									
Position at Start	AF AF/AM,AB AM AB	+3 +2 +1 0																									
Pellets	# taken (to 5)	+1 Each																									
Behavior after Pellets	NRE REM/RS RE	+2 +1 0																									
Aggress.	TG tg 0	+2 +1 0																									
Fear	Flight	-1 Each																									
Other																											
Total																											

FIGURE 4. The record form used in scoring individual cage aggressiveness or fearlessness. Each animal is tested daily and is rated in terms of its behavior toward the human observer. Such factors as vocalization, the position of the animal at the start of the test, the number of pieces of food accepted by the animal, the behavior while accepting the food, and instances of aggressive or fearful behavior are rated and summed into a single score. A partial description of this technique has been published.[4] Details concerning the scoring system and the abbreviations used are available.

would be of great importance in establishing the particular repertoire of primary and secondary reinforcers ... [or] 'motivationally relevant stimuli' " (p. 390).

To return finally to man, it may be noted that there is some direct evidence that human disorders of social behavior may be related to dysfunction within these brain structures. Terzian and Ore[12] have reported that a human subject in whom both temporal lobes had been removed showed such changes in behav-

ior as loss of fear or rage reactions that were apparently as marked as those seen in the monkey. Furthermore, Gibbs and Gibbs[13] have indicated that patients with pathological involvement of the anterior portion of the temporal lobe are much more likely to have psychiatric symptoms, in addition to their seizures, than are patients with epileptogenic involvement of other parts of the

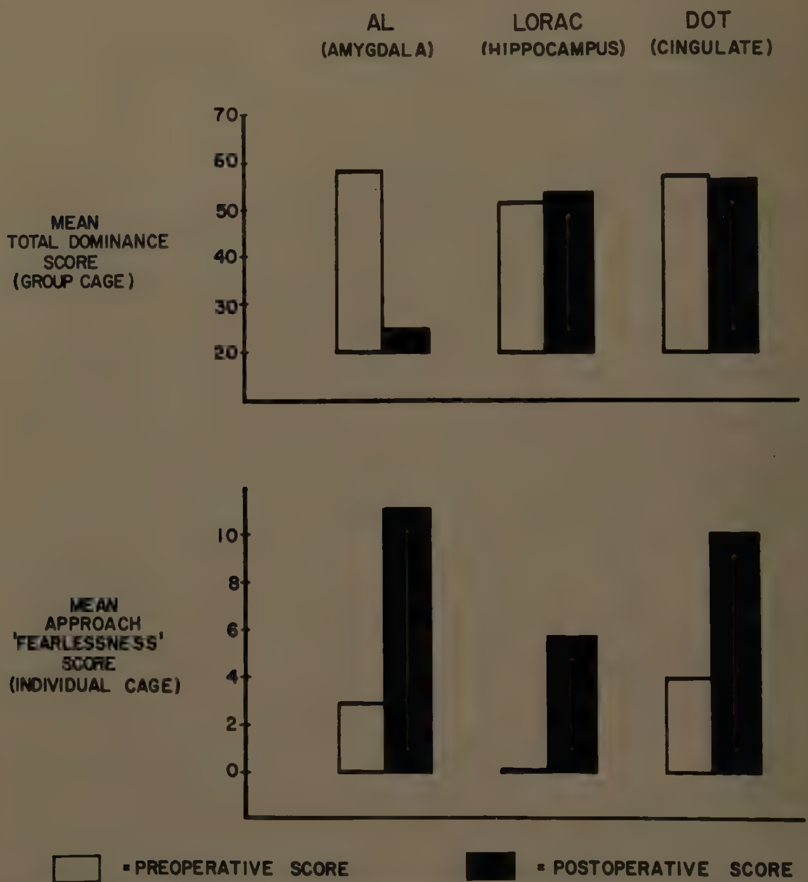
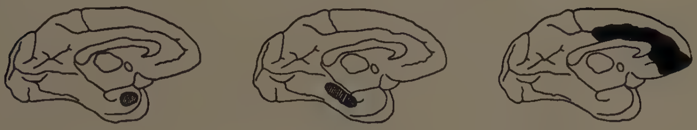


FIGURE 5. Typical effects on group and individual cage behavior obtained with lesions of the amygdala, the cingulate gyrus, and the hippocampus. The amygdala lesion produces changes in the behavior observed in both experimental situations; the other two lesions typically change only the behavior observed in the individual cage situation.

brain. These studies suggest that disordered social behavior in man and monkey may spring from involvement of the same brain structures.

Summary

Techniques for the study of the effects of the brain lesions on social behavior in monkeys are described. The use of the techniques is illustrated in relation to lesions within the limbic system of the brain. A tentative interpretation of the resultant changes in behavior, in terms of modification of cognitive and



LESION	AMYGDALA	HIPPOCAMPUS	CINGULATE
SYSTEM (PRIBRAM & KRUGER)	1st. & 2nd.	3rd.	3rd.
GROUP CAGE DOMINANCE	4↓ 1=	4=	4=
INDIVIDUAL CAGE 'FEARLESSNESS'	7↑ 1=	8↑ 1=	3↑ 2=

↑ INCREASE ↓ DECREASE = NO CHANGE

FIGURE 6. Summary of the effects on group and individual cage behavior obtained with the lesions described in this report. In each case, the changes in the scores of the operated animals were significantly greater than those of the unoperated control animals.

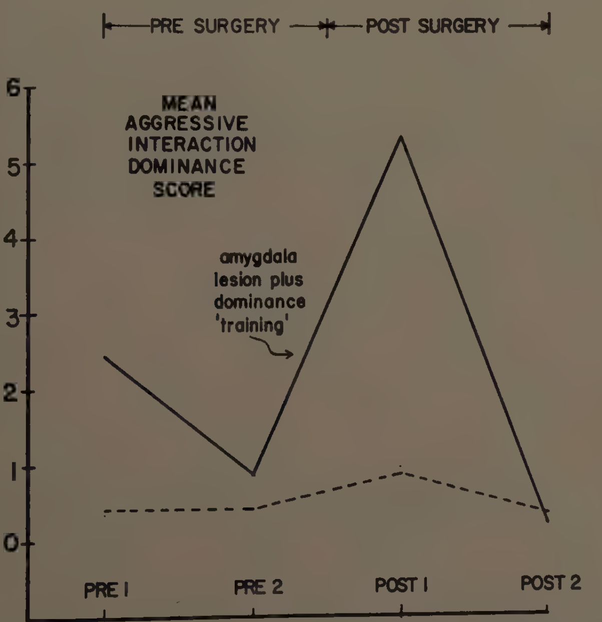


FIGURE 7. The effects of amygdalectomy and experience on aggressive behavior. Results of the experiment designed to test the hypothesis that the behavior of the amygdalectomized animal is influenced by immediate postoperative experience. Following surgery the operated animals were placed with groups of small younger animals for periods of six weeks. The figure illustrates the increase in postoperative dominance scores (interanimal aggressive acts) when the animals are returned to their original preoperative social group. The controls were animals in the same three social groups that were next below the operated animal in the dominance hierarchy. The controls had neither amygdala lesions nor postoperative "dominance" training. Additional controls in this experiment would require that animals with amygdala lesions show no upward change in dominance and/or a fall in dominance following six weeks of separation without "dominance" training, and that unoperated animals (or animals with lesions other than in the amygdala) change less than the amygdalectomized animals following "dominance" training. *Line,* 3 experimental animals; *dotted line,* 3 controls.

emotional mechanisms, is offered. It is suggested that the effects on social behavior of lesions of the limbic system, particularly within the amygdala, are to be understood in terms of the postoperative social experience of the operated animal as well as of the lesions themselves, and that the kind of brain-behavior relationships demonstrated in the monkey may have relevance to an understanding of pathological behavior in man.

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ESTIMATION OF MONKEY AGE BY DENTAL FORMULA

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Success in estimating the ages of monkeys by dental formulas depends on three factors, two variable and one invariable. Only one of the two variable factors may be under the control of the examiner; the second remains a matter of assumption in all or most instances. The third factor, which is invariable, must remain a matter of faith at all times.

The variable factor under possible control of the investigator is, of course, the number of dental inspections to which the monkey can be subjected. The variable that never can be controlled fully or evaluated fully is the health and normality of the animal. The invariable factor that must be accepted on faith is some timetable of dental development furnished by scientists who have studied in detail this particular aspect of physical growth and maturation.

For the sake of deliberate simplification of presentation, this paper will be devoted only to the problem of estimating the ages of monkeys that may be assumed to be reasonably healthy and normal. There is a large number of conditions that interfere with the usual pattern and tempo of dental-arch formation, most of which cause retardation of odontiasis, or tooth emergence. A discussion of such factors as endocrine disturbances, radiation effects, and malnutrition is not essential in a description of the methodology developed for using the teeth as a criterion of age, and consequently will be omitted.

Further simplification of presentation has been forced upon me by circumstances beyond my control. It is not possible at this time to offer a fully illustrated résumé of the complex procedure required in estimating the age of a monkey already subjected to a series of dental examinations; the basic data presupposed by such a résumé unfortunately are not yet available in published form. Therefore, the discussion will be limited to the problem of estimating age on the basis of only one dental inspection of an animal.

Without access to standards of reference of some kind it is impossible to estimate the age of any animal by its dental formula. Obviously, the most desirable standards are such as incorporate in a convenient, systematic form all that is known about the emergence of teeth in a given species. Involved in this are data on central tendency, variability, skewness of distributions, bilateral symmetry, and sex differences for each and every morphological class of teeth.

The only subhuman primate for which rather complete biometric norms have been worked out to date is the *Macaca mulatta*, or rhesus monkey. This places the macaque on a statistical par with man, and even a little ahead of him, since no universal norms have been developed as yet for the deciduous dentition of the latter. The data on the deciduous teeth and the permanent first molars of the monkey have been available for some time,[1,2] and the remaining statistics are expected to be published within the next two years. An abstract describing the character of the data on the permanent teeth of 73 normal macaques was released in 1958.[3]

As stated in 1953, "it is quite clear that attempts to 'age' a monkey with an unknown birth date by relying on a single examination of its dentition is a very poor substitute for properly spaced repeat examinations made after the acquisition of the animal. At best, a single dental inspection can furnish a relatively crude estimate of age, which is always conditioned by the extremes of the ranges of variation established for the different classes of teeth."[1]

If only one examination of the dentition can be made, it should be as detailed as possible: a mere count of the teeth will not suffice. The identity of every tooth visible in the oral cavity should be established (for example, the deciduous maxillary right first molar, and the permanent mandibular left second incisor). Any evidence of chronologic asymmetry in the emergence of a tooth and its antimere should be noted also.

By reference to a table or graph that presents the chronology of odontiasis separately for both sexes of the macaque it is usually possible to ascertain what tooth, or tooth pair, was added last to the dental arches under consideration. During the period of emergence of the premolars, which have similar central values and nearly equal standard deviations, it may be impossible in some cases to say which pair of teeth appeared last. Fortunately, in such cases the ambiguity is of little moment and will not result in appreciable errors in estimating a monkey's age.

Table 1
Emergence of Deciduous Teeth in *Macaca mulatta*

Classes of teeth		Percentiles (days)			
		Male		Female	
Max.	Mand.	2nd	98th	2nd	98th
—	di_1	0	37	0	37
di_1	—	0	40	3	41
—	di_2	0	48	3	44
di_2	—	14	66	14	66

Having determined the tooth or teeth most recently added to the dentition one may proceed to establish a tentative lower limit for the age estimate.

The most convenient figure for this purpose is supplied by a table that gives the second percentiles of normal ranges of distribution (TABLES 1 to 4). Unless some maturity indicators other than the teeth suggest that the animal is exceedingly precocious, the second percentile of emergence for the most recent tooth gives a minimal age value that has only one chance in fifty of being too high. If there is uncertainty regarding the identity of the pair of teeth last to emerge, the highest second percentile of the two or three pairs of teeth considered as possible choices should be selected as furnishing the best minimal figure.

In the rare instances in which very low limits of estimate are believed desirable, either a table supplying the first percentiles should be consulted or approximate minima be estimated from suitable frequency distribution graphs plotted on arithmetic paper or on a logarithmic probability grid. It must be

TABLE 2

EMERGENCE OF DECIDUOUS TEETH IN *MACACA MULATTA*

Male				Female			
Classes of teeth		Percentiles (days)		Classes of teeth		Percentiles (days)	
Max.	Mand.	2nd	98th	Max.	Mand.	2nd	98th
dc	—	36	95	dm_1	—	43	99
—	dc	40	99	dc	—	43	103
dm_1	—	47	99	—	dm_1	43	99
—	dm_1	51	103	—	dc	43	110
—	dm_2	113	190 (6 mo.)	—	dm_2	102	179 (6 mo.)
dm_2	—	120	212 (7 mo.)	dm_2	—	109	201 (6½ mo.)

TABLE 3

EMERGENCE OF PERMANENT TEETH IN *MACACA MULATTA*

Classes of teeth		Percentiles (years & months)			
		Male		Female	
Max.	Mand.	2nd	98th	2nd	98th
—	M_1	1–1 (395 d.)	1–9	1–1½ (416 d.)	1–6½
M_1	—	1–2 (431 d.)	1–10½	1–2½ (449 d.)	1–8½
—	I_1	2–0	2–10½	2–1½	3–0
I_1	—	2–½	3–0	2–2	2–11½
—	I_2	2–1½	3–1	2–2	3–3½
I_2	—	2–3½	3–3	2–3½	3–3½
—	M_2	2–8	3–8	2–8	3–8

TABLE 4

EMERGENCE OF PERMANENT TEETH IN *MACACA MULATTA*

Male				Female			
Classes of teeth		Percentiles (years & months)		Classes of teeth		Percentiles (years & months)	
Max.	Mand.	2nd	98th	Max.	Mand.	2nd	98th
M_2	—	2–10½	3–9½	—	C	2–7½	4–0
Pm_1	—	2–11½	4–4½	M_2	—	2–11	4–0
—	Pm_2	3–½	4–5½	—	Pm_1	2–10	4–2½
Pm_2	—	3–½	4–7	Pm_1	—	2–11	4–2½
—	Pm_1	3–1	4–7	C	—	3–0	4–7
—	C	3–2	4–9	—	Pm_2	2–10½	4–6½
C	—	3–4	5–1½	Pm_2	—	3–½	4–11
—	M_3	4–5	6–11	—	M_3	4–10	7–3½
M_3	—	4–7½	7–8	M_3	—	5–1	9–6½

pointed out that no meaningful zero percentiles for any biological phenomenon can be calculated.

The upper limit of the age estimate can be established only for a monkey that still lacks at least one tooth of its functional dental complement. Thus it cannot be established for animals that have all four third molars in the mouth; for these specimens only the lower limit can be determined.

For a tentative upper limit of age estimate it is necessary to decide what will be the next tooth, or next pair of teeth, to be added to the dentition. Again, reference to a suitable table or graph is helpful (TABLES 1 to 4). If a choice must be made between two or three different classes of unemerged teeth, the one with the lowest ninety-eighth percentile of its range of variation should be selected as that furnishing the best maximal value.

For all ordinary specimens the ninety-eighth percentile will be found satisfactory as the upper limit of the estimate, but for unusually retarded-appearing animals it may be advisable to use the ninety-ninth percentile, or an even higher value obtained from an appropriate graph. Hundredth percentiles, like zero values, are, of course, indeterminable.

The apparently inactive interval between the completion of the deciduous dentition and the beginning of permanent-tooth emergence does not create a special situation in estimating age. If the deciduous series is completed and no permanent first molar yet has pierced the gingiva, it is not possible to say, on the basis of a single examination, how long the quiescent interval has lasted. Therefore, in almost all instances the lower limit of the estimate is still furnished by the second percentile of the deciduous maxillary second molar, and the upper limit by the ninety-eighth percentile of the permanent mandibular first molar. The only exceptions are the rare cases suspected of having extremely early or late development; for these the limits must be lower or higher.

The period immediately following the addition of the fourth permanent first molar presents a similar situation, although on a smaller scale. Unless the animal is probably outside the limits of the 96 per cent range, the age estimate may be considered as between the second percentile of the maxillary first molar and the ninety-eighth percentile of the mandibular first incisor. According to TABLE 3, the period during which no visible activity whatever should be expected is about one and one-half months for the male and five months for the female.

Recapitulating the material presented, it may be said that, ordinarily, in estimating age on the basis of a single dental examination, the minimal value is given by the second percentile of the regression line summarizing the cumulative frequency distribution of emergence for the tooth added last to the dentition and the maximal value by the ninety-eighth percentile for the tooth expected to appear next.

This rule revises and extends the one given in 1953.[1]

If an investigator wishes to improve his estimate, he must keep a monkey under observation for at least a few days. He may raise the lower limit of the estimate by the number of days that the animal is under observation, and lower the upper limit by an equal amount, provided that no new tooth emerges during this period. A second examination made as much as a month later enables him to narrow the range of his estimate by two whole months.

Similar benefits may accrue from the determination of the amount of asymmetry in the emergence of a tooth and its antimere.

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CORRELATION OF SKELETAL GROWTH, EPIPHYSEAL OSSIFICATION WITH AGE OF MONKEYS*

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Introduction

Practically all rhesus (*Macaca mulatta*) monkeys used in research today must be procured from trappers in India or Pakistan. These animals are trapped for export and, of course, their ages are unknown. Since a majority of young animals are born in March, "a thumb rule" is to give all these monkeys a March birthday. Ages of these animals therefore are known only within the current monkey-crop year. For many reasons, investigators must know more definitely the limitations of physiological and psychological maturity of the animal they use for study.

For many years a variety of procedures for estimating the age of laboratory monkeys has been used. Dentition dates of deciduous and permanent teeth are documented in great detail.[1] Weight charts are relatively constant.[2] Organ weights and body measurements are well documented; however, use of these end points is time-consuming when dealing with large numbers of animals, and often it requires one or more highly trained technicians for interpretations.[3]

A few years ago this laboratory became interested in a better approach to the problem of estimating monkey age. A pilot study on bone development and maturation appeared promising. The study started in January 1958. Initially, 50 animals ranging from birth to 8 years of age were used. Subsequently, all animals born in the colony were integrated into the study at birth. As the investigation developed, aging criteria were ascertained only in those animals whose exact age was known. To date, there are aging criteria through 48 months. These monkeys and the newborn will be followed until death.

This study outlines a method of age estimation that has been developed for this monkey colony. Gertrude van Wagenen and C. W. Asling have reported a similar study. Although objectives are not identical, criteria and conclusions are complementary.[4]

Method

The animals are weighed, anesthetized with a short-acting barbiturate, positioned, and taped to the X-ray table face downward over a 14-inch by 17-inch cassette. The appendages are so positioned that the left hand and left foot are taped with a volar and plantar surface flat on the table. The right appendages are positioned so that as much of them as possible reflects the lateral position on the film. Technique varies according to thickness, and includes use of Potter-Bucky screen. It is evident that a more elaborate series of radiographic exposures would give the added advantage of more extensive coverage of views;

* The work described in this paper was supported, in part, by funds provided under Contract AF 41(657)-149 with the U.S.A.F. School of Aviation Medicine, Brooks Air Force Base, Texas.

however, this arrangement has been used in order to keep the procedure simple and inexpensive.

The bone-maturation criteria used for interpreting the age from the radiographs have been selected from this positioning of the animal. As the number of serial radiograms was increased, possible aging factors were added, changed, and deleted. Radiographs at quarterly intervals were found sufficient for development of growth criteria, even during the periods of most rapid growth (FIGURE 1).

Presently no attempt is made to catalogue bone change in animals less than six months old. A monkey this age is either born in this colony or purchased from a colony where the age is known.

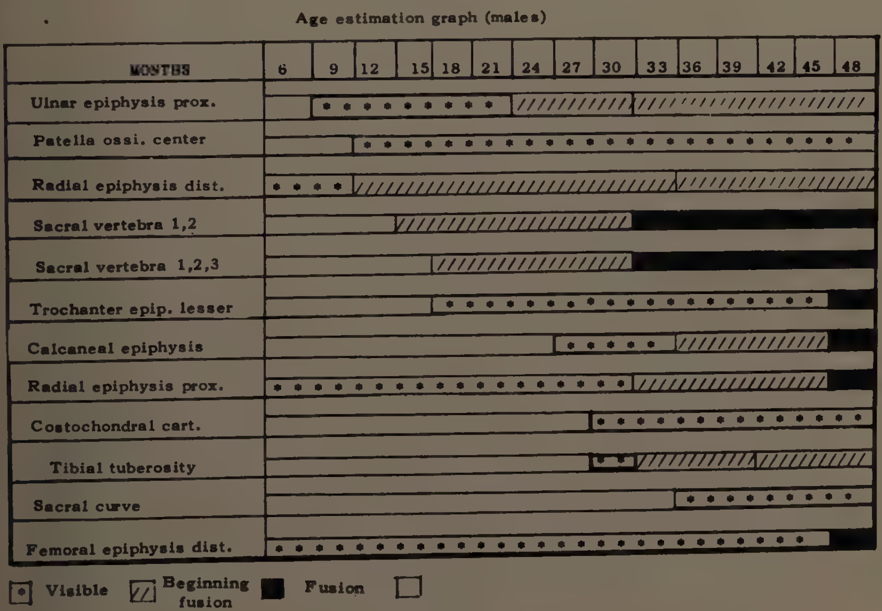

FIGURE 1.

By the sixth month the proximal ulnar epiphysis is visible. The patellar ossification center is usually visible, especially in females.

The ninth month reveals a constant patellar ossification center. The distal radial epiphysis has developed to the point of visual continuity with the shaft.

By the twelfth month the fusion of the first and second sacral vertebrae has begun. The ossification centers of the humeral head have united.

At fifteen months the second and third sacral vertebrae have coalesced. The femoral lesser trochanter epiphysis is present.

By the eighteenth month the second and third sacral vertebrae have fused. The calcaneal epiphysis is occasionally visible.

At twenty-one months the proximal ulnar epiphysis begins to fuse. In the female the first metatarsophalangeal joint sesamoid becomes visible, and the head of the radius begins to fuse.

By the twenty-fourth month the distal humeral epiphysis shows fusion. In the female the calcaneal epiphysis is visible, and the proximal ulnar epiphysis shows evidence of fusion.

The twenty-seventh month shows the costochondral cartilages definitely visible. The proximal ulnar epiphysis appears about 30 per cent united. The tibial tuberosity may become visible in females.

By the thirtieth month three sacral vertebrae have fused. Beginning fusion of distal ulnar epiphysis is visible and the proximal radius is fusing in the female.

By the thirty-third month there is fusing of the distal radial epiphysis and the calcaneal epiphysis.

The thirty-six month shows beginning fusion of the proximal fibular epiphysis, and the sacral lordotic curve is beginning to form.

By thirty-nine months the ossification center of the tibial tuberosity is generally fused to the proximal epiphysis. Fusion of the proximal radial epiphysis in the female is practically complete.

At forty-two months the distal radial epiphysis is uniting to the radius. Fusion of the proximal radius in the female is complete. A constant angulation between the second and third caudal vertebrae is seen. The distal femoral epiphysis is almost completely fused.

By the forty-fifth month fusion of the tuber calcanea, proximal radius, and lesser femoral trochanter is complete.

There is practically complete fusion of the distal femoral epiphysis and most long-bone epiphysis at forty-eight months.

Summary

Presented here is a method of estimating age in the rhesus monkey by skeletal radiograph. About thirty-five ossification changes have been used as age-estimating criteria. Females generally meet the maturation criteria 3 to 5 months sooner than males. Presently monkeys are being aged to 48 months; however, the study will be followed until complete skeletal maturation exists.

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HEMATOLOGY OF THE NORMAL MONKEY*

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Several years ago the Radiobiological Laboratory of the University of Texas and the United States Air Force started an extensive research program using the Indian *Macaca mulatta* monkey. The need for reliable information concerning the hematology of the normal monkey led to a search of the available literature. The results of this search are illustrated in TABLE 1. As shown in this table, many investigators have been interested in the macaque; however, few of these investigators worked with large numbers of animals or made large numbers of observations on a small number of animals. In many cases no reference was made to the number of animals or the number of observations leading to the cited results. In order that some more useful information concerning the hematology of the normal monkey might be obtained, a series of observations on many of the normal animals serving as controls for various experimental procedures was begun in 1953 and continued for several years.

Methods

Hematological observations were made on 538 male *M. mulatta* monkeys ranging in age from 30 to 42 months and in weight from 4 to 5 pounds. Observations in this phase extended over two years and took place at Austin, Texas, Oak Ridge, Tenn., and Los Alamos, N. Mex. All animals were maintained under routine laboratory conditions, including a standard balanced diet, medication as needed, and water ad libitum.[34]

Blood samples were collected at various times of the day, but always prior to feeding. These blood samples were obtained principally by femoral vein or arterial puncture. Some samples were collected from heel punctures, but this technique is not the method of choice with the monkey for, as the monkey ages and grows, a pad of fat is deposited under the foot skin which makes a clean puncture difficult. We have found that for all purposes blood is best obtained from puncture of the femoral vein. With practice this procedure is easily and quickly carried out with minimal trauma to the animal and technicians. Specimens obtained by femoral puncture were heparinized or sequestrated. The specific techniques employed for each analysis have been described in detail elsewhere by Krise and Wald.[17]

In the second series of observations to be discussed, twelve young male *M. mulatta* were used. These animals were controls for a larger series of experimental animals. During the first year of observation specimens were collected once a week; during the second year samples were collected every six weeks. The tests and methods used on these animals were identical with those of the first series.

* The work reported in this paper was done at the Radiobiological Laboratory of the University of Texas, and the United States Air Force, Balcones Research Center, Austin, Texas.

Results

The results of hematological analyses of the 538 male *M. mulatta* are presented in TABLE 2. Variation is the predominant feature of a heterogeneous population, such as that of the *M. mulatta* obtained from the wild in India. In all cases with the exception of one or two, the range of values is large and accompanied by a rather large standard deviation. Notable by a lack of a large standard deviation are the values for the sedimentation rate. Although the range is large, the standard deviation is low, indicating that the majority of

TABLE 1

TABULATION OF HEMATOLOGICAL DATA EXTRACTED FROM EXISTING LITERATURE

No. observ.	RBC × 10^6	WBC × 10^3	Hb	(Per cent)							Platelets × 10^3	HcT	Clotting time (sec.)	Ref.
				Poly.	Lymph.	Eos.	Mono.	Baso.	Mast.	Retic.				
10	4.64	11	75.4%	41.6	53.6	3.69			0.24					1
	6.21	5												33
1		25		40.0	60 (lymph. and mono.)									25
40		22		41.1	53.7	4.1	0.6	0.4						29
13	5.99	13	85.5%	47.4	46.5	1.0	4.9	0.2		0.3	267			18
		14												19
8	4.5–7.0	10–13		44.0–54.0	33.0–47.0	0–11.0	5.0–6.0							9
6	6.35	7	67.0%	35.8	58.0	3.5	3.4		0.3					16
8	4.94	14		42.6	51.8	2.8	1.2							10
	5.00	10	77.0%	73.0	20.0	3.0		1.0						22
27	6.43	18	12.53 gm.	35.0	61.0	2.0	2.0							2
	6.20	19		47.9	46.7	2.9	1.9	0.4						11
	6.20	15	12.2 gm.	41.5	52.0	2.1	2.4							33
11	6.20		12.86 gm.											3
3	4.94	14		38.2	59.4	1.0	0.83	0.0						31
14	4.82	16	10.47 gm.	46.9	46.5									7
19	5.20	15		37.7	58.2					0.6	475	40.0		20
19	5.20	15	12.2 gm.	36.0	59.0	0.5	3.0	0.5			475		78	26
2	5.28	13	11.37 gm.											8
13	5.69–6.30	16	14.1 gm.	31.9	62.0	3.2	2.7		0.2					23
5	4.17–6.07											35–45		27
2	5.0–6.0	10–15												6
4	5.68	19		43.9	56.0									24
4	5.32	14	12.2 gm.	52.5	43.0	1.0	3.0	0.5		0.7	332	39.9		30
26	5.23	10	13.2 gm.	50.2	42.2	4.9	0.75			1.85	306			28
59	5.76	15	13.59 gm.	48.3	47.9	2.2	1.61					44.98		21
10	4.0–5.5	10–25	14.0–15.5 gm.											4
10	4.81	13	13.89 gm.											5
10										1.03				12
	5.28	15	13.39	43.46	52.07	2.26	1.66				475	42.5	78	9
15	6.38	17	12.95	49.5	45.3	2.2	2.5	0.1						14
22		18		10,820	7,000									15

values falls near the mean. In practice, I have found that the sedimentation rate is an accurate indicator of the state of health of the animal.[17] As an animal sickens and even before clinical symptoms of disease are manifest the condition will generally be foreshadowed by an increase in sedimentation rate. Any increase of more than 5 mm. per hour is an indication of a sick monkey.

The results of long-term observations on the *M. mulatta* are shown in FIGURES 1 to 5. These observations were made over a period of two years, start-

TABLE 2*

TABULATION OF HEMATOLOGICAL ANALYSES OF 538 *MACACA MULATTA* MONKEYS

Study	No. of obser-vations	Mean	S.D.	Range
Red blood cell count (cells/min.³)	876	5,571,900	729,780	3,110,000–8,570,000
White blood cell count (cells/mm.³)	891	15,155	5,981	1200–43,100
Hemoglobin (gm./100 cc.)	655	11.72	3.025	7.0–16.5
Polymorphonuclear leukocytes (per 100 cells)	738	35.79	16.70	4–91
Lymphocytes (per 100 cells)	738	60.52	17.26	7–95
Monocytes (per 100 cells)	738	0.717	0.379	0–8
Eosinophils (per 100 cells)	738	2.63	2.37	0–16
Basophils (per 100 cells)	738	0.213	0.178	0–4
Bilobed lymphocytes (per 1000 lymphocytes)[13]	439	0.589	0.415	0–12
Stabs (per 100 cells)	738	0.37	0.31	0–9
Hematocrit (heparinized or sequestrated)	392	37.01	6.75	12–51
Hematocrit (citrated)	42	45.47	3.85	35–53
Platelets (dry method)	119	211,744	68,100	79,900–368,640
Platelets (wet method)	57	344,035	71,750	250,000–750,000
Clotting time (1-tube)	27	1 min. 55 sec.	39 sec.	1 min.–3 min. 15 sec.
"Clotting time" (resistance)	161	4.32 min.	0.36 min.	1.5 min.–9.4 min.
Sedimentation rate (per hour)	448	0.902 mm.	2.2 mm.	0.0–28 mm.
Reticulocytes (per cent)	76	0.354	0.187	0.1–3.4
Plasma prothrombin time (sec.)	203	13.18	2.54	10.5–18.5
Serum prothrombin time (sec.)	174	54.12	23.57	19.1–180
Electric resistance rate (milliohms/min.)	132	5.67	2.21	0.2–13.9

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ing in January 1954 and extending to January 1956. The observations are still in progress, but the results of only the first two years are being reported, since early in 1956 the animals were inadvertently fed an overdose of vitamin D and suffered from hypervitaminosis D. The results of observations on the hematology during and after that period are difficult to interpret, and the animals could not be considered "normal."

Because of the recent interest in seasonal variations in animal behavior, both social and biological, one perhaps would expect to find seasonal variations in the hematological values of the monkey; however, this is not the case, or at least there does not appear to be any consistent seasonal trend over the two years of observations reported here. As shown in FIGURES 1 to 5, there

is no definite change with time that might be called cyclic or seasonal in any of the parameters measured.

When this series of observations was started, it was felt from experimental evidence of shorter duration (8 weeks) that 5 ml. of blood could be withdrawn weekly without causing any great change in the hematological picture (unpublished data). It was expected that a certain minimal stimulation of the marrow would result in a gradual increase in certain parameters, but no drastic or acute changes were anticipated. However, if one considers the reticulocyte

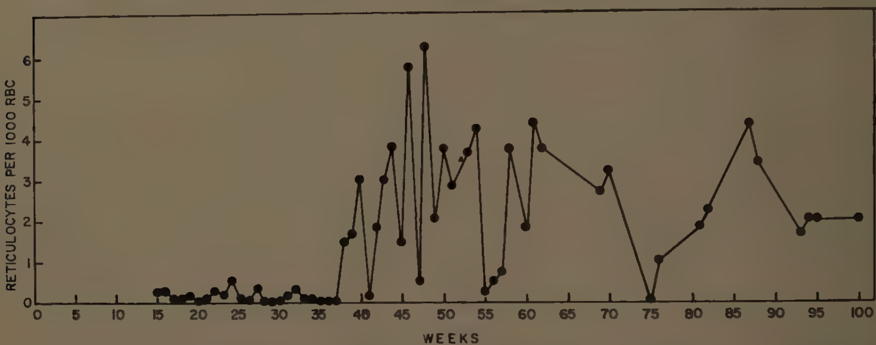

FIGURE 1. Average weekly reticulocytes per 1000 red blood corpuscles of 12 *M. mulatta* for a period of 2 years.

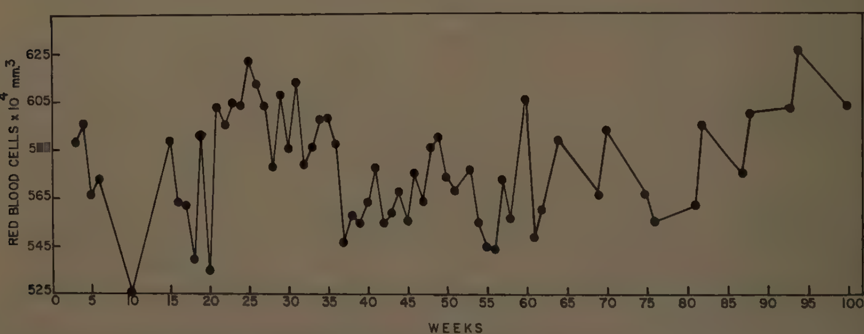

FIGURE 2. Average weekly red blood cell count of 12 *M. mulatta* for a period of 2 years.

picture for this two-year period (FIGURE 1) a rather consistent picture of low counts until the twentieth week of weekly bleedings is seen; then it becomes apparent that the continual chronic blood loss has resulted in a marrow stimulation, as shown by the increase in reticulocyte count.

The red blood corpuscle count for this two-year period shows much variability (FIGURE 2). However, there seems to be an increase of red blood cells at about the twentieth week that persists until about the thirty-seventh week. This increase corresponds to the period of increased reticulocyte appearance.

As indicated in FIGURE 3, there is little real change in the mean corpuscular hemoglobin concentration, mean corpuscular volume, hematocrit, mean corpuscular hemoglobin, and hemoglobin. In all of these parameters there is

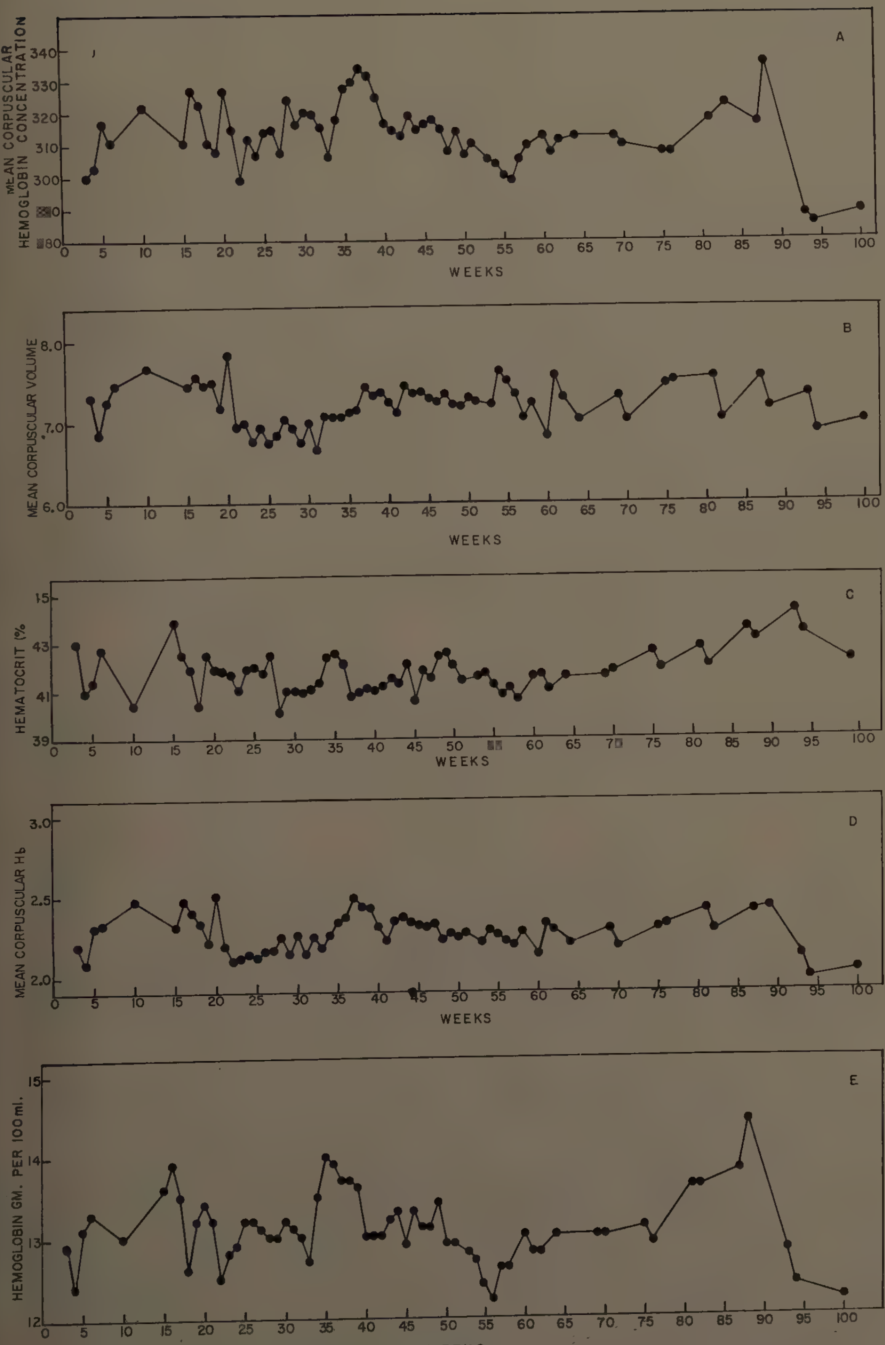

FIGURE 3. *A*, average weekly mean corpuscular hemoglobin concentrations of 12 *M. mulatta* for a period of 2 years. *B*, average weekly mean corpuscular volume of 12 *M. mulatta* for a period of 2 years. *C*, average weekly hematocrit percentage of 12 *M. mulatta* for a period of 2 years. *D*, average weekly mean corpuscular hemoglobin of 12 *M. mulatta* for a period of 2 years. *E*, average weekly hemoglobin (gm. per 100 ml.) of 12 *M. mulatta* for a period of 2 years.

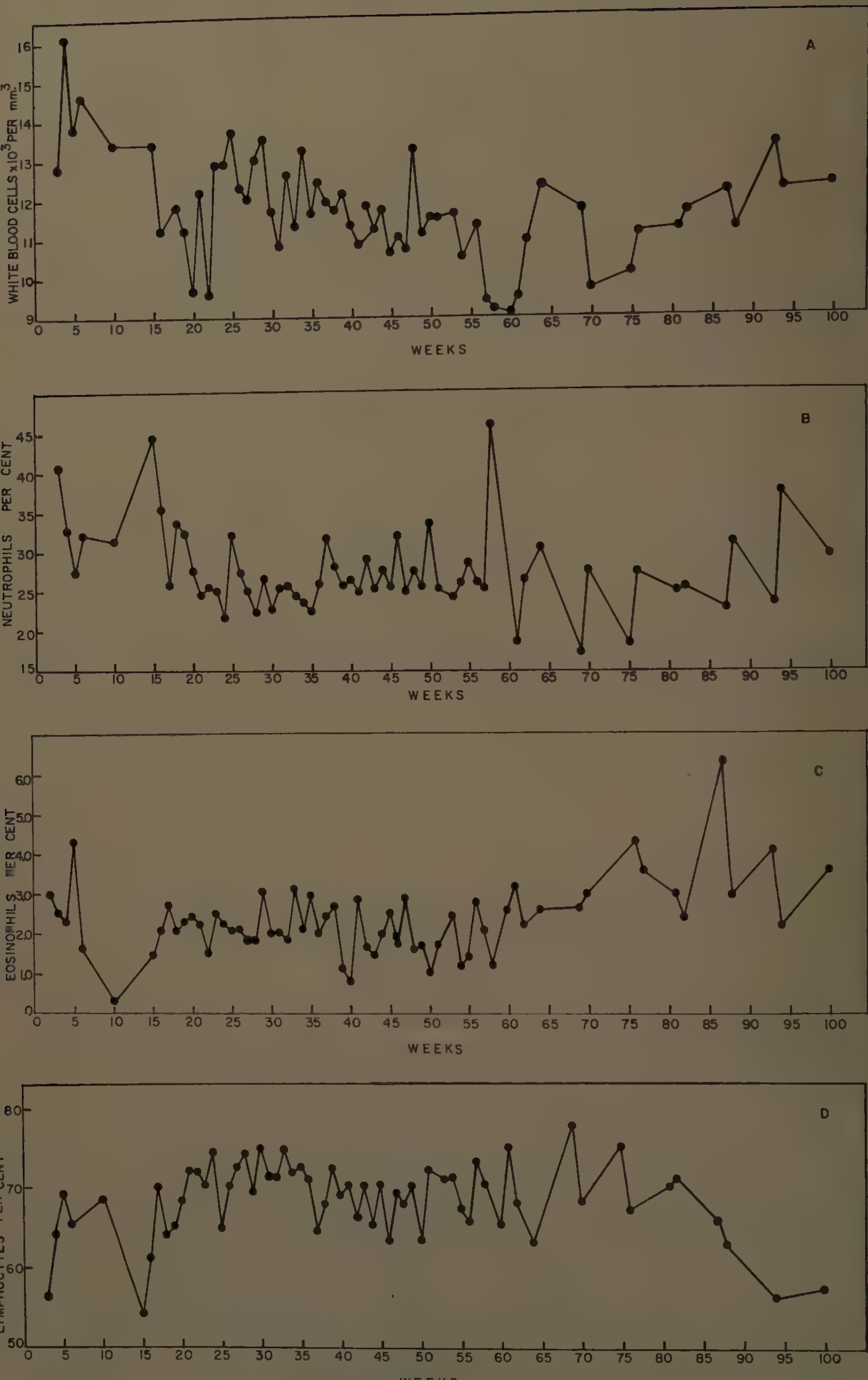

FIGURE 4. *A*, average weekly white blood cell counts of 12 *M. mulatta* for a period of 2 years. *B*, average weekly neutrophils (percentage) of 12 *M. mulatta* for a period of 2 years. *C*, average weekly eosinophils (percentage) of 12 *M. mulatta* for a period of 2 years. *D*, average weekly lymphocyte (percentage) of 12 *M. mulatta* for a period of 2 years.

considerable variation around a mean that seems to be rather consistent over the two-year period. There are no pronounced changes corresponding to the reticulocyte response.

In the leukocyte series, represented by FIGURE 4, there is an initial decrease in the white cell count, neutrophil count, and eosinophil count, followed by a long period of more or less constant counts with only slight variation. The lymphocyte count goes up initially, in contrast to the decline in neutrophils. This would be expected in the normal animal, as there is a definite balance between the number of lymphocytes and neutrophils.

The platelet count of monkeys is extremely variable and is therefore difficult to interpret in the normal animal. The platelet count for two years is shown in FIGURE 5. The variations shown are well within the normal range.

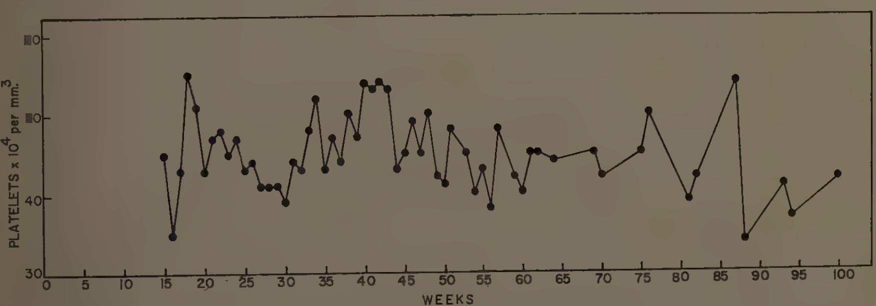

FIGURE 5. Average weekly platelet count of 12 *M. mulatta* for a period of 2 years.

Discussion

These observations concerning the hematology of the normal *M. mulatta* are presented not as absolute, but as representing the best efforts of a large laboratory to control methods, feeding, housing, and disease of a number of monkeys in order to obtain the best data available. It appears that the monkeys imported from India represent a heterogeneous population that demonstrates the expected variability of biological measures.

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ELECTROCARDIOGRAPHIC STUDIES IN THE *MACACA MULATTA* MONKEY*

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In attempts at the experimental production of rheumatic fever in the *Macaca mulatta* (rhesus) monkey, the electrocardiogram was used as a guide in determining evidence of cardiac tissue alteration. In previous studies on monkeys Lloyd[1] and De Waart and Storm[2] presented observations on the classic leads; Ruskin and Rigdon[3] described one chest lead "corresponding to CF_4 in man" in addition to the classic leads. Our pilot experiment on a group of unselected monkeys indicated the need for a more complete study of the electrocardiogram in the normal animal in this species. This report presents the results of a study using the classic leads, the augmented unipolar limb leads, and three chest leads in a group of apparently normal rhesus monkeys.

Materials and Methods

Animals. Forty-four apparently normal, unanesthetized *Macaca mulatta* monkeys weighing from 1.7 kg. to 3.7 kg. and averaging 2.3 kg. were studied. The sexes were almost equally divided. The criteria for normality were: (1) absence of clinical illness (among others, two old tuberculin 0.1 strength intradermal eye skin tests were made) and (2) normal or negative values of at least 3 serial determinations of corrected sedimentation rate, white blood cell count, including a differential count, and blood and stool cultures.† There was no evidence of murmurs or abnormal sounds at auscultation of the heart or on the phonocardiograms of the animals. Approximately 10 monkeys were later sacrificed for use of their kidney cells. Their hearts showed no gross pathology and no histopathological changes other than the normal spectrum of alterations described by Vanace elsewhere in this monograph. Each animal was housed in an individual cage and fed commercial monkey biscuit and water ad libitum.

Methods. The monkeys were strapped to a specially adapted restraining board and carefully placed in the true supine position. All tracings were made after a 10-minute rest period. Serial electrocardiograms of unanesthetized animals in the supine position were obtained with a Sanborn Stethocardiette and a Sanborn Twin-Beam Cardiette at a speed of 75 mm./second. The records were so standardized that a current of 1 mv caused a deflection of 1 cm. The effects of anesthesia on the electrocardiogram were observed in 7 animals before and after intravenous administration of Nembutal (approximately 10 to 20 mg./kg.).

* The work described in this paper was supported in part by Research Grant A-2942 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, Bethesda, Md.

† Normal values: Corrected sedimentation rate (Westergren method): less than 2 mm./hour; white blood cell count: 10,000 to 25,000 per cu. mm.; differential count: less than 50 per cent segmented neutrophils and less than 5 per cent bands and immature forms; blood cultures: negative; stool cultures: negative for enteric pathogens.

Leads. In addition to the three standard leads, the augmented unipolar limb leads and three unipolar precordial leads were taken. The chests of a number of monkeys were mapped to determine the sites best suited to record the potentials of the right ventricle, septum, and left ventricle. By trial and error we found that these potentials were usually registered best for the right ventricle (MV_1)* in the fourth right intercostal space, 4 cm. from the mid-

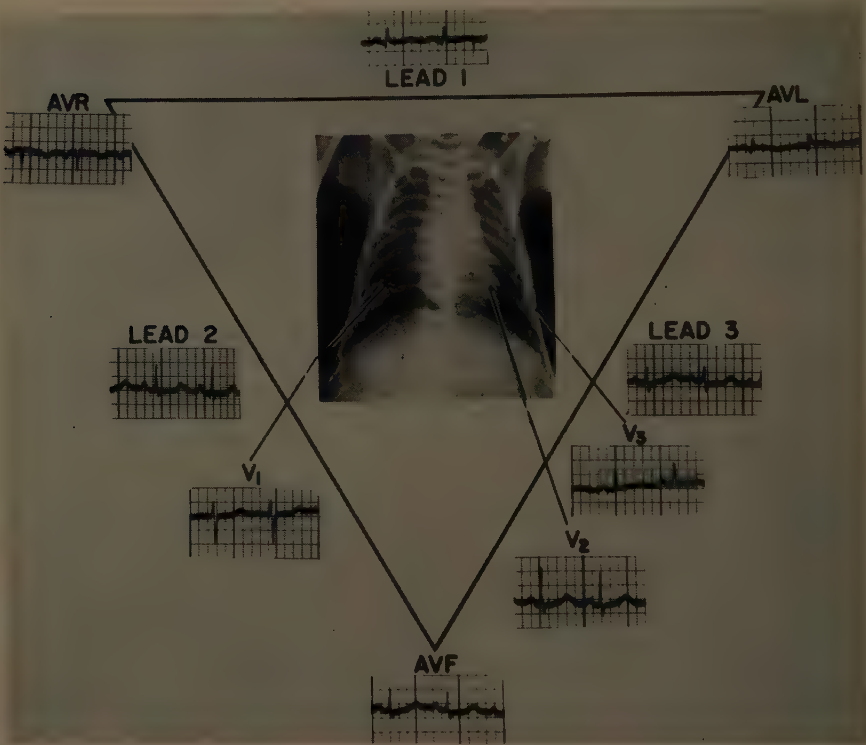

FIGURE 1. Typical electrocardiogram of normal *M. mulatta*, superimposed on an AP chest film of same animal. Classic leads are represented on sides of triangle of Einthoven and augmented unipolar limb leads at its vertexes. Location of precordial leads is indicated by the dots on the chest film. The similarity in morphology with the human electrocardiogram is evident. In this record, MV_2 does not register septal potentials, but chiefly, left ventricular potentials, and the transitional zone is situated between MV_1 and MV_2.

sternal line. The septal potentials (MV_2), were registered best on the left side of the chest in a point symmetrical to MV_1, and the left ventricular potentials (MV_3) were registered best at the left mid-axillary line in the fifth intercostal space, approximately 1 cm. below the level of MV_2 (FIGURE 1).

Calculation of the axes. The mean manifest axes of P, QRS, and T were calculated from their areas in two different classic leads, and were plotted in a triangle of Einthoven, as described by Sodi-Pallares and McCalder.[4]

* The precordial leads were labeled MV (Monkey V) leads to avoid confusion with the human precordial leads, which are taken at different sites.

Results

FIGURE 1 presents the usual morphology of the electrocardiogram of the normal *M. mulatta.* TABLE 1 summarizes the findings in 128 analyzed tracings. The rhythm was sinus in all records and no arrhythmias were observed. The heart rate ranged from 160 to 333/min., with an average of 260/min. in the unanesthetized monkey. Serial tracings taken from the same animal on different days showed no essential variations. The P-R intervals ranged from 0.047 to 0.100 sec., averaging 0.064 sec. The average duration of QRS was 0.027 sec., with a range from 0.020 to 0.036 sec. The Q-T intervals ranged from 0.107 to 0.200 sec., with an average of 0.148 sec. The mean axis of QRS projected on the frontal plan was +55° and varied from −41° to +127°. ÂP was +73°, with a range from +29° to +99°, and ÂT was +72°, ranging from +34° to +90°. There were no significant changes in these measurements in relationship to weight.

TABLE 1
SUMMARY OF NORMAL MONKEY ELECTROCARDIOGRAPHIC DATA*

Measurement	Range	Average†
Weight (kg.)	1.7–3.7	2.3
Rhythm	Sinus	Sinus
Rate	160–333	260
P-R interval	0.047–0.100	0.064
QRS duration	0.020–0.036	0.027
Q-T interval	0.107–0.200	0.148
ÂP‡	+29° to +99°	+73°
ÂQRS	−41° to +127°	+55°
ÂT	+34° to +90°	+72°

* A total of 128 tracings taken from 44 monkeys was analyzed.
† Arithmetic averages, calculated from the mean values of the data of serial tracings on each monkey rather than directly from the isolated tracings.
‡ The axes of only 27 monkeys were calculated.

P wave. The P wave in L_1 was found to be unusually low and occasionally flat and, in one instance, it was biphasic. In L_2 and L_3 the P wave was always upright. In aVR it was usually inverted, occasionally biphasic, but never upright. In aVL it was usually flat or biphasic. In aVF, MV_2, and MV_3 it was always upright. In MV_1 it was usually upright, but in one instance it was biphasic.

QRS complex. According to the electric position of the heart, different QRS patterns could be registered. With the exception of aVR, in which a Q-S pattern usually was obtained, the deepest Q wave was seen in aVL, where it varied from a small q to a Q-S pattern although it was of less magnitude than that shown by aVR. Of the classic leads, the deepest Q was registered in L_2, where it always measured less than one fourth of the length of R and on one occasion was as deep as 3 mm. Among the precordial leads a small q could be registered in MV_3, occasionally in MV_2, but never in MV_1. The tallest R was usually registered in L_2; its highest value was 25 mm., although

in the great majority of records it did not surpass 15 mm. In one instance the tallest R wave was registered in MV_2; it was 26 mm.

T wave and ST segment. The T wave was usually low and occasionally flat in L_1. In rare instances it was inverted in L_3. It was always inverted in aVR and occasionally in aVL. In all other leads it was always upright. The ST segment was usually isoelectric (not deviating more than 0.5 mm. from the base line). In one instance it was elevated 0.8 mm. in L_2.

Alterations of the complexes due to positioning. Early in this study it was noted that a change from the true supine position to any degree of lateral decubitus produced significant changes in the complexes. When the animal was placed in a semilateral decubitus position (at a 45° angle with the board)

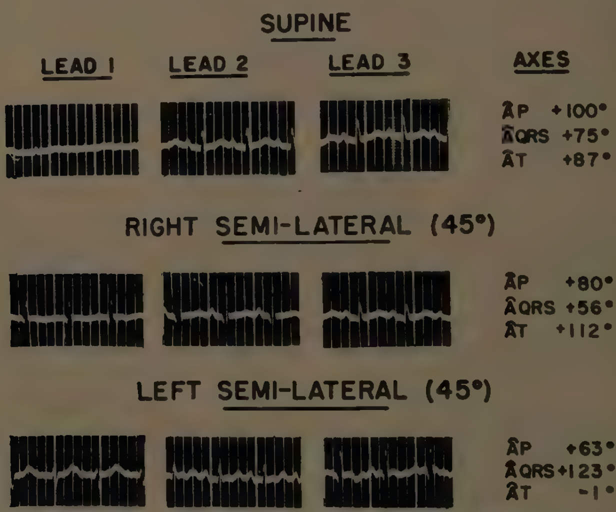

FIGURE 2. Influence of positional changes on the electrocardiogram of the normal monkey. Note the marked change in morphology of the complexes and deviation of the various axes when the monkey is changed from supine position to right and left semilateral decubitus positions.

a marked rotation of ÂQRS and ÂT and a minor change in ÂP were noted (FIGURE 2). When this angle on the right was increased to approximately 60 to 70° (FIGURE 3), a deep Q with an inverted T often was noted. This last change simulates the findings seen in old anterior myocardial damage, and might be interpreted as such by an observer unfamiliar with the effect of position on the monkey electrocardiogram.

Discussion

The studies herein reported yielded preliminary observations on the electrocardiographic patterns of 44 apparently normal *M. mulatta* monkeys. The electrocardiogram of the monkey was found to be similar to that of man, excluding the rate and proportionally shorter intervals. The Einthoven law was observed. Serial tracings taken from the same animal over periods as long as 6 months showed little variation. TABLE 2 summarizes all previous

studies known to us of the normal electrocardiogram of the monkey. Although in general our results agree with these previously recorded studies, some of our findings, especially with reference to intervals and the mean mani-

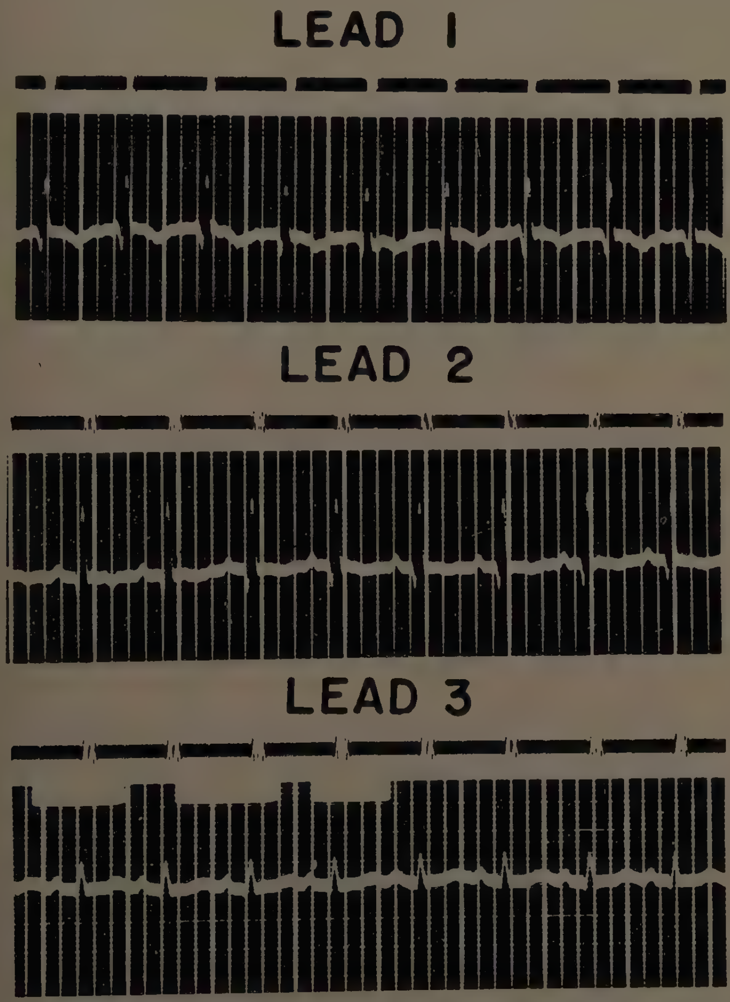

FIGURE 3. The same monkey as in FIGURE 2, but lying in a more pronounced (approximately 70°) right semilateral decubitus position. Note the deep Q wave (almost half the length of R) and inverted T wave in L_1 and L_2, suggesting old anterior myocardial damage.

fest axis of QRS, differ to some degree. The various intervals obtained in our studies were in general usually shorter than those previously reported. This applies particularly to the duration of QRS, which never exceeded 0.036 seconds. Our range of the mean manifest axis of QRS from -41 to $+127°$ was greater than reported previously. The majority of animals, however, had an ÂQRS of from $+30$ to $+90°$, and only 4 monkeys showed a negative axis of

TABLE 2

NORMAL ELECTROCARDIOGRAM OF MONKEY

Review of the Literature

Authors	Monkey species	No. of monkeys	Weight (kg.)	Leads	Type of anesthetic	Results						
						Rhythm	Heart rate (per min.)	P-R interval	Duration of QRS	Q-T interval	Manifest axis of QRS	T wave in L_1
Lloyd[1]	*M. mulatta* *M. irus*	17 3	Not stated	Standard	4 animals, sodium amyl-ethyl barbiturate; 16, ether	Sinus	160 to 250	0.064 sec. to 0.104 sec. Average 0.077 sec.	Not stated	Not stated	Not stated	Upright
De Waart & Storm[2]	*M. irus*	12	2 to 7	Standard	Penoctum or Evipan sodium I.V. (0.2 to 0.3 cc. of a 10% sol.)	Sinus	171 to 256 Average 225	0.054 sec. to 0.088 sec. Average 0.073 sec.	0.020 sec. to 0.044 sec. Average 0.028 sec.	0.140 sec. to 0.220 sec. Average 0.172 sec.	+50 to +75° Average 63°	Always upright
Ruskin & Rigdon[3]	*M. mulatta*	27	2 to 7	Standard, and a bipolar chest lead corresponding to CF_4 in man	Not stated (no anesthesia?)	Sinus	170 to 280	0.060 sec. to 0.100 sec.	0.020 sec. to 0.050 sec.	0.140 sec. to 0.200 sec.	+50 to +115°	Inverted in 1 and flat in 9 tracings

QRS. Our findings of a considerable change in unanesthetized monkeys in the morphology of patterns according to the position of the animal in relation to the restraining board should be emphasized. De Waart and Storm[2] noted a similar phenomenon, but to a much lesser degree. These positional alterations in complexes may be due to the relative lack of fixation of the heart in the mediastinum of these young animals. We have noted at autopsy that the heart shifts markedly in the mediastinum with change of position.

The augmented unipolar limb leads and multiple precordial leads in the monkey to our knowledge have not been reported previously. The augmented unipolar limb leads in the monkey in general resemble those of man. More often than in man aVR records pure intracavitary potentials. A deep Q and an inverted T wave in aVL are common findings. In man and monkey the same variations are shown by aVF.

In most of our precordial leads MV_1 and MV_3 respectively showed right and left ventricular potentials; that is, a small r followed by a deep S in the former and a small q followed by a tall R in the latter. The R of MV_3 occasionally was followed by a small s. In a few instances MV_3 registered a transitional pattern, that is, an R followed by an S of equal magnitude. The transitional zone in the precordium varied considerably, but it was more commonly found at a point 4 cm. from the midsternal line, in the fourth left intercostal space, where MV_2 was recorded.

A low or flat T wave in L_1 was not significant per se, since it was usually low in this lead and occasionally flat. In De Waart's and Storm's series the T wave was always positive in this lead. In Ruskin's and Rigdon's[3] series the T was flat in L_1 in nine instances and inverted in one. In CF_4 the T was flat in two instances and inverted in two. In our precordial leads T was always upright.

Most recorded studies by others were made on anesthetized animals. To determine what effect, if any, anesthesia would have on the tracings we took records of 7 animals anesthetized with intravenously administered Nembutal (10 to 20 mg./kg.). Except for a retardation of the rate and proportionally shorter intervals, no essential changes were noted in these records when compared with previous tracings taken on the same animals without anesthesia. It was of interest that a variable amount of body tremor, which was quite evident on records obtained with the sensitive Twin-Beam Cardiette on unanesthetized animals, was much reduced or abolished with anesthesia.

These preliminary studies have been of value in establishing base-line data for our experimental work. We plan to make further studies of the normal monkey that will include direct cardiac tracings and intracavitary recordings in order to understand more fully the normal excitation of the monkey heart. In addition, we hope to produce pathological cardiac alterations by a number of methods and to study their sequential electrocardiographic changes.

Summary

The normal electrocardiographic pattern of the *Macaca mulatta* (rhesus) monkey was determined as a base line for studies on experimental rheumatic fever. A total of 128 tracings, taken from 44 normal monkeys weighing from 1.7 kg.

to 3.7 kg. were analyzed. With the animals in the supine position, serial electrocardiograms were obtained with a Sanborn Stethocardiette and a Sanborn Twin-Beam Cardiette. The classic leads were taken on successive days for a determination of the variations in individual monkeys. The classic and augmented unipolar limb leads and 3 unipolar precordial leads were taken subsequently from many of the animals in the true supine and in both the semilateral decubitus positions. The alterations in the various complexes and direction of the axes due to positional change are mentioned.

The results, including the average pattern, rate, and rhythm, different intervals, duration of the QRS complex, and direction of the different axes, are tabulated and discussed with reference to studies previously reported.

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SPONTANEOUS AND INDUCED MALIGNANT NEOPLASMS IN MONKEYS

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Early reports of spontaneous neoplasms in wild animals noted an apparent paucity of such lesions in certain groups, particularly in monkeys.[1-3] Furthermore, some attempts to induce neoplasms in monkeys apparently have not met with the success that similar attempts have achieved in other species. These observations suggest that monkeys may be more resistant generally to the development of neoplasms than man and other species. Any resistance to the development of neoplasms in monkeys would be of interest in itself and also would influence their usefulness in the laboratory study of neoplasms. The accumulation of further pertinent evidence in recent years is sufficient to warrant a re-examination of these earlier observations.

Spontaneous Neoplasms

The forty-nine cancers that have been reported in monkeys since 1885 when Bland Sutton noted the first cases have many similarities to cancer in man. The wide variety of neoplasms noted include most of the same types seen in man (TABLE 1). Furthermore, the organ system most commonly involved is the digestive tract of men and monkeys. The close anatomical resemblance is also striking. The limited information available on many of the reported cases makes an evaluation of their natural history difficult.

In cases that have been carefully studied, however, the resemblance to man is evident. A carcinoma of the cervix recently reported by Hisaw and Hisaw is an excellent example.[22] This tumor developed in an old monkey. It infiltrated the adnexae, rectum, and bladder and obstructed the ureters, resulting in bilateral hydronephrosis. It metastasized to the regional lymph nodes and the lungs. This pattern of growth is the usual one seen in untreated carcinoma of the cervix in humans. The metastatic pattern in 3 of the carcinomas of the tongue and the malignant lymphoma is also similar to that seen in man. The incidence of metastasis, 14 of the 49 cancers, is not as high as one would expect with the same type of tumors in humans. However, the true incidence of metastasis in these cases cannot be determined, as the autopsies were often not complete or no statements were made as to the presence or absence of metastasis.

The over-all incidence of neoplasms in groups of monkeys followed in menageries and research laboratories has been quite low. An early study by Fox at the Philadelphia Zoological Garden revealed the incidence of neoplasms in primates to be the lowest of any mammalian order.[3] Later reports by Fairbrother and Hurst of 600 monkey autopsies and by Kennard of an additional 246 without a single neoplasm support this impression.[37,38] In comparing the incidence of neoplasms in monkeys to man or to other species, there are a number of factors known to influence the frequency of neoplasms that must be considered. A most important and obvious factor is that of age. The

TABLE 1
SPONTANEOUS MALIGNANT NEOPLASMS REPORTED IN MONKEYS

Type & location of neoplasms	Animal	Age (years)	Sex	Metastasis	Author
Squamous cell carcinoma of mouth	*Macaca mulatta*	Adult	M	Regional nodes	Zuckerman[4]
Squamous cell carcinoma of tongue	*Macaca mulatta*	16	M	No	Steiner *et al.*[5]
Squamous cell carcinoma of tongue	*Macaca irus*	14	M	Regional nodes	Steiner *et al.*[5]
Squamous cell carcinoma of tongue	*Macaca mulatta*	13.5	M	Regional nodes	Klüver & Brunschwig[6]
Squamous cell carcinoma of tongue & buccal mucosa	*Saimiri sciurea*	8	F		Klüver & Brunschwig[6]
Squamous cell carcinoma of tongue	*Macaca mulatta*	25	M		Krotkina[7]
Squamous cell carcinoma of tongue	Macaque				Hemmens[8]
Squamous cell carcinoma of esophagus	*Macaca fuscatus*	15.5*	M	Regional nodes	Ratcliffe[9]
Carcinoma of stomach	*Macaca mulatta*	Adult	M	Yes	Klüver[10]
Carcinoma of small bowel	Ringtail monkey				O'Conner[11]
Adenocarcinoma of pancreas	*Papio cynocephalus*	18*	F	No	Ratcliffe[9]
Adenocarcinoma of pancreas	*Cercopithecus aethiops sabaeus*	13.8	F	Liver	Ratcliffe[9]
Adenocarcinoma of gall bladder	*Papio papio*		F		Lombard & Witte[12]
Adenocarcinoma of gall bladder	*Papio papio*	23.8	M	No	Fox[13]
Adenocarcinoma of rectum	*Macaca sinica*			Yes	Ratcliffe[14]
Carcinoma of renal pelvis	*Macaca mulatta*	16	F		Antonov[15]
Adenocarcinoma of kidney	Cacajao				Plimmer[16]
Hypernephroma of kidney	*Cebus apella*			Lymph nodes, lungs, adrenals	Scott[17]
Hypernephroma of kidney	*Macaca mulatta*	28.7	M	No	Ratcliffe[14]
Hypernephroma of kidney	*Macaca mulatta*	16.6	F	Widespread	Ratcliffe[14]
Hypernephroma of kidney	*Macaca mulatta*	10.2	M	No	Ratcliffe[14]
Hypernephroma of kidney	*Macaca mulatta*	10.1	M	No	Ratcliffe[14]
Malignant adenoma of kidney	*Macaca mulatta*	27*	M	No	Vadova & Gelshtein[18]
Hypernephroma of adrenal	*Cebus apella fatuellus*	4.2	M	No	Fox[3]
Adenocarcinoma of prostate	*Macaca mulatta*	Old	M	No	Engle & Stout[19]
Fibrosarcoma of breast	*Cercopithecus aethiops sabaeus*				O'Conner[11]
Adenocarcinoma of breast	*Macaca mulatta*	11	F		Vadova & Gelshtein[18]

TABLE 1—*Continued*

Type & location of neoplasms	Animal	Age (years)	Sex	Metastasis	Author
Adenocarcinoma of breast	*Macaca mulatta*	Old	F	Regional nodes	Lombard[20]
		Adult			
Squamous cell carcinoma of cervix uteri	*Macaca sinica*		F		Fox[21]
Squamous cell carcinoma	*Macaca mulatta*	Old	F	Lymph nodes, lungs, & local invasion	Hisaw & Hisaw[22]
Adenocarcinoma of ovary	*Macaca irus*	13*	F		Fox[23]
Squamous cell carcinoma of skin	*Macaca maurus*	9.1*			Ratcliffe[24]
Carcinoma of lung	*Saimiri sciurea*		M		Lombard & Witte[12]
Adenocarcinoma in epicardium	*Cebus apella*		F	No	Iglesias & Lipschutz[25]
Osteogenic sarcoma of ulna	*Papio comatus*	9.1	M	Lungs & heart	Ratcliffe[26]
Osteogenic sarcoma mandible	*Macaca mulatta*	Adult	F	No	Bagg[27]
Osteosarcoma of radius	*Lemur catta*	5*	F	No	Warwick[28]
Sarcoma of humerus	*Macaca mulatta*	3	F		Vadova & Gelshtein[18]
Osteosarcoma of maxilla	*Macaca mulatta*	3*	M	No	Kent & Pickering[29]
Multiple myeloma of bone	Monkey			Lungs, liver, & lymph nodes	Oshima[30]
Sarcoma of scalp	Cercopitheque				Plimmer[31]
Sarcoma	*Lemur*				Plimmer[32]
Glioma of eye	*Macaca radiata*	Young			Sutton[33]
Carcinoma pituitary & optic chiasm	Baboon				Sutton[33]
Gliosarcoma of brain	*Papio doguera*	6*	M	No	Vadova & Gelshtein[18]
Melanoma of peripheral nerve	*Macaca mulatta*	5.5*	M	No	Blokhin *et al.*[34]
Hodgkin's sarcoma	Baboon				Kelley[35]
Malignant lymphoma	*Macaca mulatta*	6*	F	Liver, kidney, bone marrow, spleen	Kent & Pickering[29]
Cancer	Mozambique				King[36]

* Years in captivity.

association of age to the incidence of neoplasms is well known in man, as well as in other species (FIGURE 1).

An examination of the age of the monkeys listed in TABLE 1 that developed malignant neoplasms suggests that there is also a strong association in the monkey between age and the development of neoplasms. Most of the monkeys reported in the literature were captured when quite young. As their life span in captivity is short due to exposure to human diseases to which they are quite susceptible and due to sacrifice for experimental purposes, relatively few reach the age group in which the incidence of tumors in man and other species is high. Hence, groups of monkeys from which the incidence of tumors might

be calculated are heavily weighted with young animals. The expected incidence for this reason alone would be very low. A recent report by Lombard and Witte from the Philadelphia Zoological Garden shows the importance of age to the incidence of neoplasms in monkeys.[12] From 1901 to 1935, the average exhibition period for primates was 24 months and the incidence of malignant tumors 0.7 per cent. From 1935 to 1955, the average exhibition period for primates, 46 months, was almost doubled. This was apparently due to improvement in their diet. During this period, the incidence of tumors, 2.0 per cent, increased almost 3 times.

There are other factors that may contribute to the scarcity of neoplasms seen in captive monkeys. The effect of natural selection in eliminating animals before capture with cancers such as neuroblastoma, retinoblastoma, and

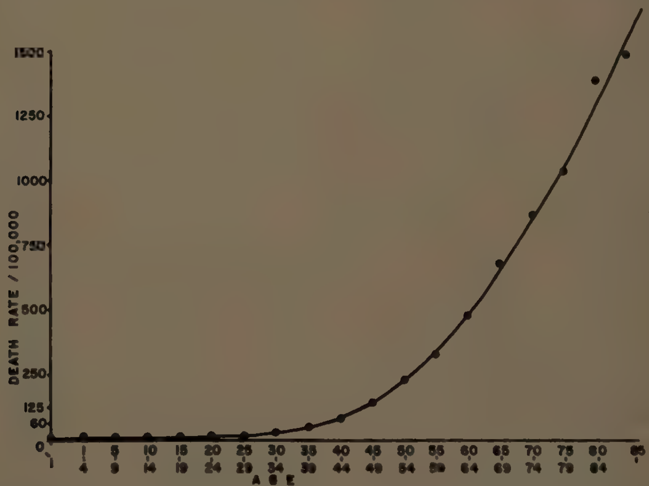

FIGURE 1. The incidence of malignant neoplasm for various age groups in the United States for 1957.[39]

Wilm's tumor that occur early in life cannot be measured, but should be noted. Differences in environmental carcinogens may also be of some importance. Monkeys are not ordinarily exposed to the industrial carcinogens to which some groups of men have been, nor to such possible carcinogens as cigarette smoke. When these considerations, particularly the age of the animals, are allowed for, the apparent paucity of neoplasms in monkeys is not as impressive. The evidence that monkeys have a wide variety of neoplasms that look and act like neoplasms in other species is much better founded and is probably more significant. A continued effort to collect detailed clinical and autopsy records for monkeys is needed in order to resolve this problem.

Induced Neoplasms

Two approaches have been used to produce neoplasms in monkeys. First, neoplasms, usually of human origin, have been implanted in monkeys and, second, monkeys have been exposed to agents known to be carcinogenic in

some other species. The transplanted neoplasms were placed in the subcutaneous tissue or the anterior chamber of the eye. Fresh tissue or bacteria cultured from cases of Hodgkin's disease have been tried most often.[40-47] However, these transplants did not survive. Other human cancers have also been used, but without success.[48-50] A few attempts to transplant neoplasms arising in one monkey to other monkeys are recorded.[26,27,34,51] A "take" with a squamous cell carcinoma of the tongue was reported in one animal in this group.

TABLE 2
EXPERIMENTS TO INDUCE NEOPLASMS IN MONKEYS

Investigator	Carcinogen	Animals	Results
Overholser & Allen[53]	Castration, cervical trauma & theelin	9 Monkeys	Atypical hyperplasia cervix uteri
Engle & Smith[54]	Estrin	4 *M. mulatta*	Squamous metaplasia cervix uteri
Zuckerman[55]	Estrin	2 Monkeys	Squamous metaplasia cervix uteri
van Wagenen[56]	Theelin	5 (Male) *M. mulatta*	Hyperplasia and metaplasia GU tract
Engle *et al.*[57]	Estradiol	5 *M. mulatta*	Hyperplasia of endometrium, metaplasia of cervix
Vargas[58]	Castration & estradiol	4 *M. mullata*	No neoplasms
Iglesias & Lipschutz[25]	Estrogens	5 *Cebus apella*	Cystic hyperplasia of endometrium, metaplasia cervix uteri
Pfeiffer & Allen[59]	Estrogen, methylcholanthrene, benzpyrene, dibenzanthracene	50 *M. mulatta*	Metaplasia of mammary ducts and cervical glands, hyperplasia of epidermis
Bonne *et al.*[60]	Gas-works tar	20 *M. cynomolgus*	Lesions suggesting carcinoma—regressed spontaneously
Lushbaugh[61]	Motor lubricating oil	9 *M. mulatta*	Adenomata of stomach and colon resembling carcinoma—lesions regressed
Sugiura *et al.*[62]	Petroleum derivative	6 *M. mullata*	All developed papillomas; three histologically malignant
Petrov *et al.*[63]	Radium (2.0 to 5.5 μg.)	7 *M. mullata*	Osteogenic sarcomas in three
Krotkina & Baradze[64]	Radium (8.9 to 21.0 μg.)	16 Monkeys	Four osteogenic sarcomas
Kent & Pickering[29]	Ionizing irradiation	120 *M. mulatta*	Three malignant tumors

It should be noted that other species react in much the same way to implanted neoplasms unless pretreatment with cortisone or X irradiation is used.[52] Thus, failure of these tumor implants to grow in monkeys does not set them apart from other species.

Estrogen or some derivative of estrogen with or without castration or cervical trauma constituted the stimulus most frequently used in attempts to induce neoplasm in monkeys (TABLE 2). A typical hyperplasia of the cervix was often noted, but no evidence of neoplasms was seen. Pfeiffer and Allen's study involving exposure of 50 monkeys to estrogen, methylcholanthrene, benzpyrene, and dibenzanthracene over a ten-year period is particularly noteworthy

despite the fact that neoplasms were not produced.[59] The variation in sensitivity of different species to specific carcinogens is well known. Therefore, the failure of a species to respond to one group of carcinogens does not necessarily indicate a general resistance to carcinogens.

Exposure of monkeys to other carcinogens has been more promising. Bonne *et al.* noted skin lesions that histologically resemble carcinoma following the application of gas-works tar to the skin of monkeys.[60] The lesion did not metastasize and regressed spontaneously. In monkeys exposed to motor lubricating oil Lushbaugh described adenomatous changes in the stomach and colon that resembled adenocarcinoma.[61] These lesions also regressed spontaneously and did not metastasize. More recently Sugiura *et al.* exposed the skin of 6 monkeys to a petroleum derivative MH 101 over a period of 9 years.[62] Papillomas developed on all 6 animals. The lesions in 3 of these histologically appeared malignant. Metastases were not found. The exposures to the petroleum derivative were continued until each animal died. Whether the lesions would have regressed on removing the irritant is not known. Additional studies with this petroleum derivative would be useful.

Ionizing irradiation is a most promising agent for inducing tumors in monkeys. Three different goups of investigators have exposed monkeys to ionizing irradiation under conditions that permitted the animals to live for several years after exposure. Malignant tumors were found in each case at different time intervals following the exposures. The first report was by Petrov *et al.* in 1951.[63] Seven monkeys received intramedullary implants of radium (2.0 to 5.5 μg.). Three of these animals developed osteogenic sarcomas at the implantation site about 10 years later. Krotkina and Barabadze reported a similar experiment in 1957 in which 16 animals received intramedullary implants of higher doses of radium (8.9 to 21.0 μg.).[64] Four of the 16 developed osteogenic sarcomas at the implantation site from 1 year, 11 months, to 5 years, 2 months later, the increase in dose being associated with a shorter latent period.

A recent summary of the results of 18 years of work by these 2 groups notes 2 additional osteogenic sarcomas for a total of 9 induced with radioactive substances.[65] One additional malignant neoplasm following the intramedullary implantation of polycyclic hydrocarbons was reported. An abundance of distant metastases was described in these animals. This constitutes the first report of experimentally induced neoplasms that metastasized in monkeys. The 120 monkeys included in the report by Kent and Pickering in 1958 received focal irradiation to the eye or head as a part of a program designed to study cataract formation in monkeys following ionizing irradiation. Only 18 of these were in groups receiving a dose sufficiently high (2000 r, 2500 rep., or 3000 r) and at the same time surviving sufficiently long for at least one member to develop a malignant neoplasm. Most of the animals received relatively low doses. One group received a higher dose, but died within a few days after the irradiation. Three of the 18 developed malignant tumors, 2 fibrosarcomas, and 1 glioblastoma multiforme in the area of irradiation after 474, 854, and 892 days. Surviving members of this group, as of July 1, 1959, had not developed additional neoplasms (R. J. Young, personal communication).

The use of focal ionizing irradiation for further studies, designed to induce

neoplasms in monkeys, offers several advantages. First, the above data strongly suggest that ionizing irradiation is carcinogenic in monkeys. Second, directing the irradiation to small areas permits the animals to survive for the long periods necessary for the tumor to develop. As the latent period for tumor development apparently varies with the dose of irradiation, it may be possible to shorten appreciably the latent period by increasing the dose. Also, it may be possible to expose more than one small area in each monkey and thus lower the total number of animals needed for an experiment.

Summary

A variety of spontaneous neoplasms, including most of the types seen in man, has been reported in monkeys. The appearance and natural history of these neoplasms closely resemble their human counterparts. The incidence of neoplasms in captive monkeys appears to be lower than that in man. This does not necessarily indicate a resistance of monkeys to the development of neoplasm. The short life span of monkeys in captivity and other factors that have been mentioned provide a more likely explanation. Additional detailed clinical and autopsy data on monkeys are needed to clarify this question. Numerous attempts to implant or induce neoplasms in monkeys have failed; others have resulted in lesions that resemble but do not act like malignant neoplasms. Ionizing irradiation, which is known to be carcinogenic in many species, has been reported by three groups to result in the development of malignant neoplasms in monkeys. Additional experiments designed to induce neoplasms in monkeys and to study the natural history of these neoplasms are needed.

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DIURNAL PATTERNS OF MICTURITION AND DRINKING IN RHESUS MONKEYS*

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Although numerous studies of water balance have been made in man and in several species of animals, investigations of a similar nature on subhuman primates have been almost completely lacking. Studies on the monkey appear to be limited to the report by Krohn and Zuckerman (1937), who investigated water balance in one pigtailed macaque monkey (*Macaca nemestrina*) during three menstrual cycles. Their data were utilized by Richter (1938) in his calculations of water intake in monkeys in comparison with other species and have been cited as applicable to monkeys in general (Adolph, 1943; H. Smith, 1951).

In the present investigations the patterns of water intake and urinary output were analyzed in twenty monkeys (*Macaca mulatta*) as the first step in a study of alterations of bladder function resulting from experimental lesions in the central nervous system. A preliminary report on the findings in ten of these animals was presented briefly by Smith and Feldmahn (1954).

Methods

The monkeys (*Macaca mulatta*) used in these studies were all mature healthy members of a colony kept for experimental purposes. They had been in captivity for varying lengths of time, from 6 months to more than 1 year, and had never previously been subjected to any experimental procedure. They were considered to be "adapted" to their mode of existence. For study, they were removed from the colony and placed in a large cage in a separate room, where disturbing factors were reduced to a minimum, and the room was entered usually only once a day. An occasional animal never became adjusted to the new environment and jumped vigorously whenever approached. Accurate records could not be obtained on such animals and hence they were not used for this study. Observations on the patterns of micturition and drinking were made on 20 monkeys ranging in weight from 3.1 to 9.6 kg. Sixteen of these were females; two were pregnant. Female animals were chosen more often than males for studies of bladder function in order to facilitate subsequent investigations entailing repeated catheterizations.

The recording room, situated off the main corridor in the animal house, was not specially insulated against sound, and hence the experimental animals were subjected at times to various noises such as the periodic barking of dogs. Occasionally another monkey could be seen or heard by the experimental animal. Temperature of the room was maintained at 70° to 80° F. The humidity varied from 50 to 65 per cent. Most of the monkeys were studied for a period of from 2 to 4 weeks prior to operative procedures that were performed after a definite

* The work described in this paper was supported by Research Grants B-162 and B-1399, from the Institute of Neurological Diseases and Blindness, Public Health Service, Bethesda, Md.

pattern of voiding and drinking had been established for each monkey. A few animals were studied for longer periods, and observations were sometimes made at repeated intervals. Monkeys often reacted to being placed in the cage by eating and drinking very little for several days and by becoming excited when the room was entered. Signs of excitement and fright were usually accompanied by voiding, but this reaction did not occur after the monkey had become accustomed to the cage and to the caretaker.

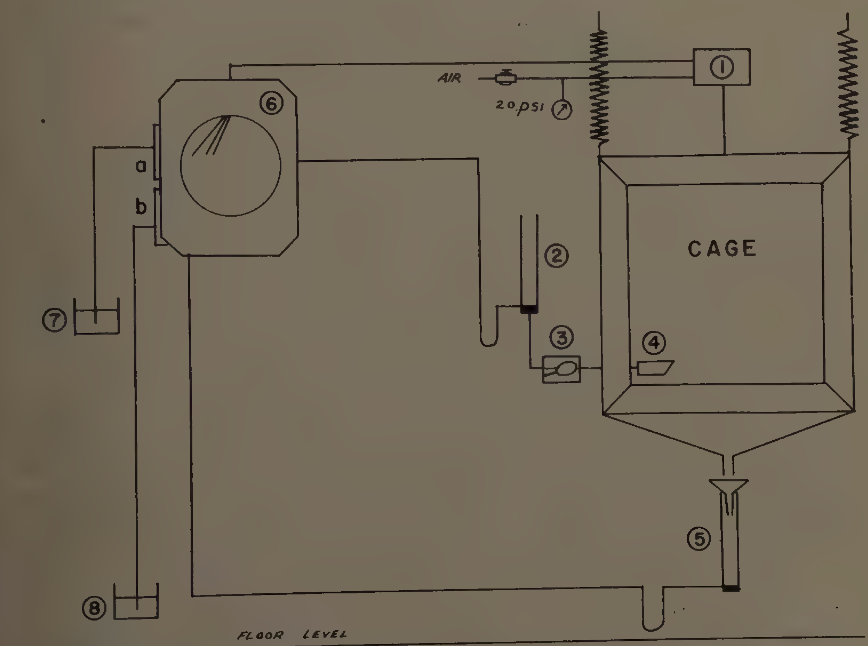

FIGURE 1. Diagram of activity cage and recording system: (1) special apparatus for transmitting movement of cage to recording pen; (2) water burette; (3) constant-level reservoir with float valve; (4) drinking cup; (5) urine burette; (6) manometers (*a*, *b*) with 24-hour chart; (7) reference water level for water burette; and (8) reference water level for urine burette.

The experimental cage and the recording equipment are represented diagrammatically in FIGURE 1. The cage itself, measuring 50 in. in each dimension, was constructed of half-inch wire mesh on a steel frame and was equipped with a sliding safety plate-glass panel on one side so that the animal could be photographed in the cage. The glass was protected by a wire mesh screen that ordinarily was in place behind it and that could be removed when photography was undertaken. A small sliding door on one side gave access to the interior for feeding the animal and for cleaning the floor. A slanting removable metal tray funneled the urine into a glass burette placed under the cage.

In order to record graphically the activity of the monkeys, the cage was suspended at the corners by coil springs of appropriate elasticity so that any movement of the animal resulted in movement of the cage. This movement was

transmitted by a rod connecting the cage to a special apparatus so designed that the movement was registered on the same 24-hour clock chart that served to record the water intake and the urinary output. This method of recording activity did not make it possible to quantitate activity accurately, but it gave adequate representation of the relative amount of movement at different times of the day, the pattern for each animal being quite uniform from day to day.

The recording apparatus used in this investigation was specially designed to register on a 24-hour chart the exact time and the volume of each drink and each voiding. The records were usually changed each day, and food was placed in the cage at that time. Occasionally the chart was left on for longer periods of time. Diet consisted of a weighed quantity of food pellets, adjusted to the amount the monkey would consume in 24 hours. Drinking water was made available at all times.

The method of recording urine output utilized an aneroid manometer in a water-filled system so designed that variations in pressure were recorded directly as volume of fluid by a moving pen writing on a 24-hour clock chart calibrated from 0 to 350 ml. or 0 to 500 ml. The urine receptacles consisted of specially designed glass burettes calibrated to contain 350, 500, 700, or 1000 ml. in a height of 20 in., the appropriate size for each animal being selected after determining the daily output for a few days. Since the writing pen was adjusted to read zero when the burette was empty, any increase in the height of the column of liquid was registered on the chart, thus giving the exact volume voided as well as the time of voiding.

The method of recording water intake utilized the same general principle as that employed for recording the urinary output, but certain modifications were necessary. The writing pen was adjusted to read zero when the water burette was full; hence any decrease in the height of the column of water was registered on the chart. Thus the pens recording water intake and urinary output moved in the same direction across the chart, and the volume changes occurring at any time could be read directly. The water level in the drinking cup inside the cage was maintained by connecting it to the water burette through a constant-level reservoir containing a float valve. When the water level in the drinking cup was lowered, water flowed into the cup from the reservoir and the float fell and opened the valve, permitting water to flow from the burette, thus restoring the former level in the cup. The resulting decrease in pressure of the column of water due to its diminished height was immediately registered on the recording chart and could be read off as volume, thus giving the time and amount of each drink.

The charts used in the recording equipment show at a glance the daily pattern of drinking and voiding (FIGURE 2). For tabulation purposes the data were transcribed on weekly sheets giving the total water intake and urine output for each 24-hour period beginning at midnight. The days of the week were noted on the tabulation sheets because the activity and noise in the animal house were at a minimum over the week end; thus Saturday and Sunday served as a control as to whether the noise of dogs being fed or men working early in the morning had any effect on the pattern of voiding and drinking. A period of 7 consecutive days was chosen from each monkey's record and subjected to detailed analysis.

Results

Micturition. In analyzing the results of these investigations, the data of which are summarized in TABLE 1, it was found that monkeys have a diurnal pattern of micturition that is similar in most of the animals studied and remarkably constant for any one individual. The fundamental pattern is characterized by a large voiding early in the morning occurring about the same time each

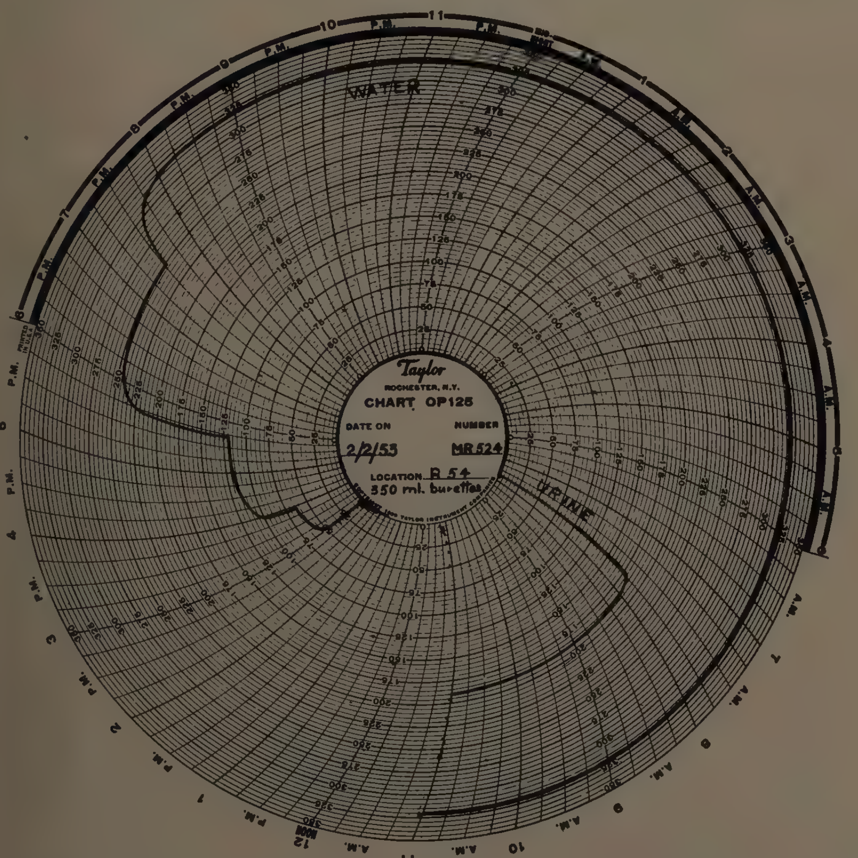

FIGURE 2. Record of monkey MR 524, male, 8.5 kg., showing a single 190-cc. voiding at 7:45 A.M. and total water intake of 320 cc. taken in 4 drinks between 3:00 P.M. and 7:30 P.M. Activity not recorded.

day and no voiding during the night. Most monkeys had one or more small voidings during the day in addition to the large morning voiding, but only 2 of the 20 monkeys studied (MR 539 and MR 5318) were consistently found to void more frequently in the daytime, 11 to 23 times. An occasional monkey voided only once a day with periods of 22 to 26 hours between voidings (FIGURE 3). The mean frequency of voiding for all the monkeys studied was found to be 4.5 per day. Even the monkeys with the highest frequencies did not void dur-

ing the night, the nonvoiding period in these animals averaging from 10 to 12 hours. The mean nonvoiding period for all the monkeys was 17.8 hours. The volumes of the first morning voiding expressed as percentages of the total daily urinary output were found to represent from 45 per cent to 100 per cent of the daily total, except for the 2 monkeys with a pattern of frequent daytime voidings, in which the first morning voiding accounted for a smaller fraction of the total daily output.

TABLE 1
MEAN DAILY VALUES FOR URINE OUTPUT AND WATER INTAKE
(One-Week Periods)

Monkey	Weight (kg.)	Month	First morning voiding			Frequency of voiding	"Nonvoiding" interval (hours)	Urinary output per 24 hours (ml.)	Urinary output per sq. m. (ml.)	Water intake per 24 hrs. (ml.)	Water intake per sq. m. (ml.)	Food intake (gm.)
			Median time E.S.T.	Volume (ml.)	Per cent of total output							
5115 PF*	6.1	Jan.	7:20	188	74	3.0	18.2	255	652	420	1070	100
524 M	8.5	Feb.	4:55	203	54	4.6	15.6	378	776	445	914	210
531 M	4.5	Nov.	6:30	219	67	4.1	18.6	329	1030	421	1320	170
533 F	3.8	June	5:30	88	51	6.6	17.6	171	598	310	1080	85
538 F	3.8	Mar.	8:05	102	46	5.0	16.0	220	769	415	1450	100
539 F	4.1	July	5:30	44	13	15.7	10.6	342	1140	532	1770	85
5312 F	3.5	May	7:15	125	79	2.6	19.7	158	587	228	848	100
5313 M	9.6	June	4:30	225	45	7.7	13.2	504	953	811	1530	300
5314 F	4.5	Mar.	6:55	143	54	3.4	18.3	265	831	488	1530	110
5316 F	6.2	Mar.	6:50	134	50	4.1	17.5	269	679	442	1120	100
5317 F	6.0	Mar.	8:00	163	44	3.4	18.9	371	961	447	1160	100
5318 F	4.3	April	5:10	100	24	14.7	12.6	410	1330	710	2300	120
5319 F	4.8	Dec.	10:00	105	100	1.0	22.7	105	316	191	575	50
5320 F	5.6	Nov.	5:45	270	83	2.1	20.3	324	880	505	1370	120
5321 F	5.7	Nov.	6:30	230	84	2.0	19.8	274	735	410	1100	155
5322 F	6.5	Dec.	8:35	163	95	1.1	23.7	171	420	271	666	90
5323 PF	6.7	Jan.	7:15	236	72	2.1	20.3	329	793	371	894	100
563 F	3.1	Dec.	7:00	92	77	1.7	18.8	120	482	267	1070	145
5611 M	4.0	June	5:00	78	60	3.2	14.2	129	437	342	1160	155
5615 F	3.3	Jan.	7:45	90	78	2.6	19.1	116	446	171	658	100
Means	5.2			150	62	4.5	17.8	262	741	410	1179	125

* PF = pregnant female, M = male, F = female.

The uniformity of the daily pattern of voiding and drinking for the individual monkey is illustrated in FIGURE 4, which gives the record of 5 consecutive days for a large male monkey, MR 524, weighing 8.5 kg. The volumes of the first morning voidings in this animal ranged from 198 to 225 ml. voided between 3:45 A.M. and 5:00 A.M. There were 2 to 4 additional voidings during the day, none after 3:00 P.M. A similar pattern of voiding was found in a female monkey, MR 5115, who was 5 months pregnant at the time of the observations (FIGURE 5).

The change of seasons with increasing amount of daylight in the spring appeared to have some effect on the time of the first morning voiding in most of

the animals. Five of 6 monkeys studied between April and September had a median time for first voiding earlier than 6:00 A.M. (TABLE 1). Twelve of 14 monkeys studied between September and April had a median time of voiding later than 6:00 A.M., but some monkeys, such as MR 524, showed a pattern of very early morning voiding, 3:45 to 5:00 A.M. in the winter (FIGURE 4). No special effort was made to change the relationship of light and darkness in these

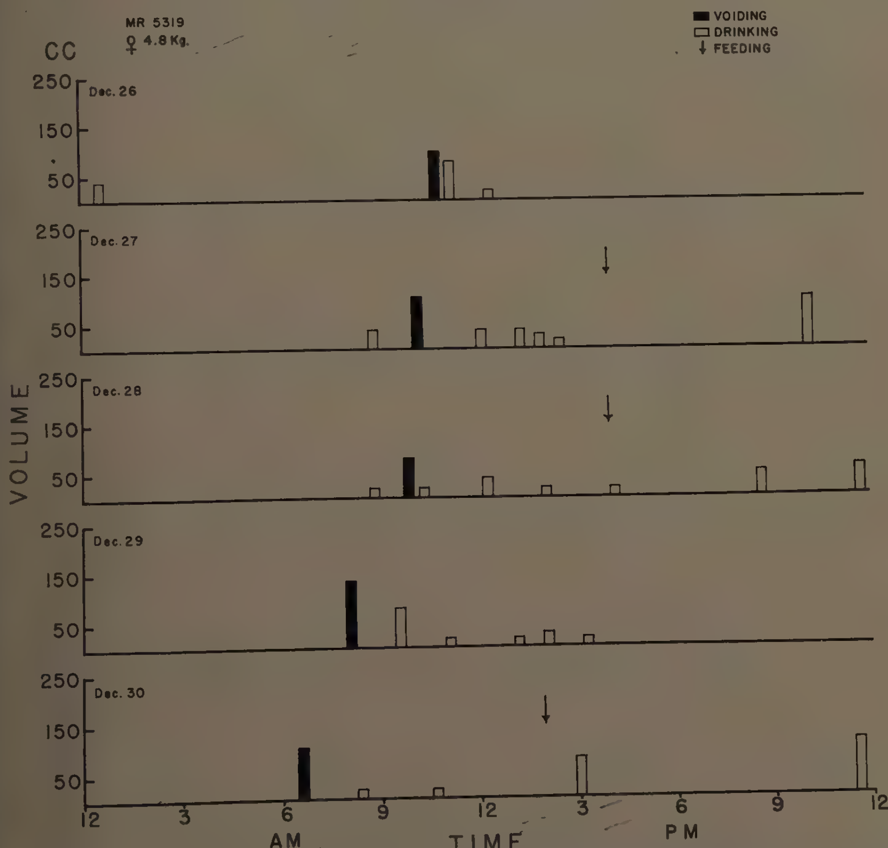

FIGURE 3. The pattern of voiding and drinking for 5 consecutive days in a female monkey (MR 5319, 4.8 kg.) that voided only once each 24 hours. The arrow indicates the times when food was put into the cage and the chart changed.

investigations, but it was observed that when the lights were accidentally left on throughout the night, the pattern of micturition was not affected. Altering the time at which the charts were changed and food put into the cage did not seem to result in any changes in the pattern that remained almost identical from day to day. The fact that the pattern of voiding was quite similar in different monkeys and appeared to be characteristic for the individual monkey is well illustrated by the records of animals that were restudied several times after long intervals. One of these animals (MR 539), when first studied in 1953, showed a

pattern of multiple voidings in the daytime varying from 11 to 23 per day and, when studied again 3 years later, was found to void with almost the same frequency.

Drinking. Patterns of drinking were found to be quite variable, not only in comparison of one monkey with another, but also in the same monkey from day to day. In some of the animals, drinking occurred in the course of several

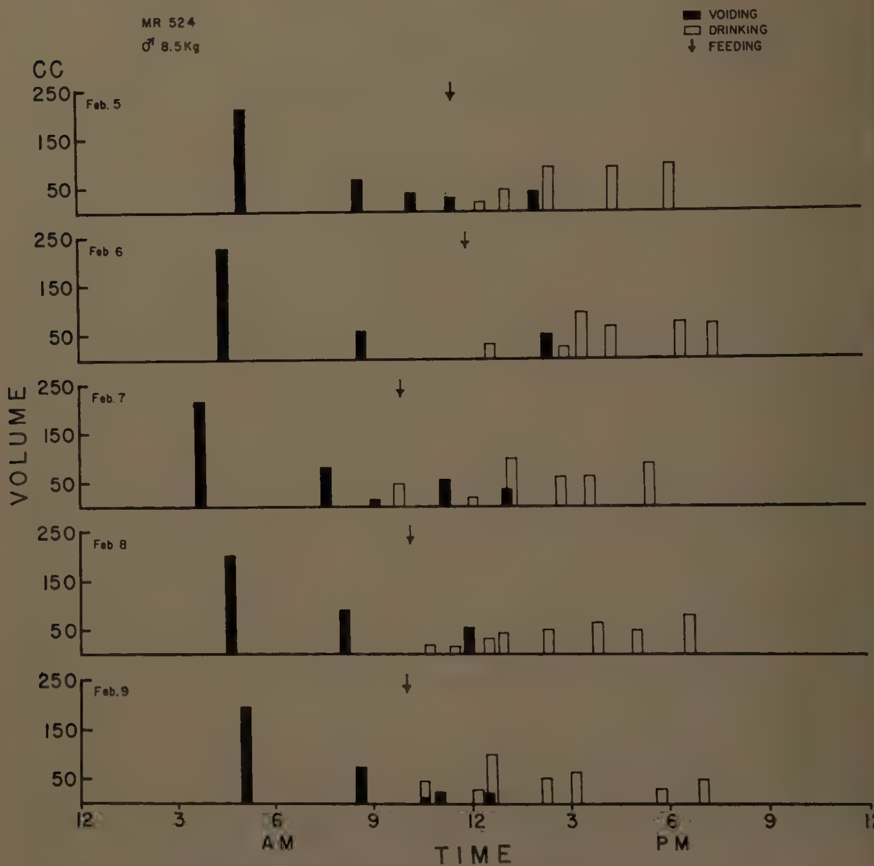

FIGURE 4. Patterns of voiding and drinking for 5 consecutive days in a large male monkey (MR 524, 8.5 kg.). The arrow indicates the time when food was put in the cage and the chart changed.

hours during the day as illustrated in FIGURE 4, where it can be seen that all of the water ingestion took place between 10:00 A.M. and 7:30 P.M. In other monkeys, drinking was scattered throughout the day with considerable variations in the size of the individual drinks which ranged from 10 to 180 ml. A number of monkeys regularly took large drinks of water during the night, as illustrated in FIGURE 6. This chart indicates that the monkey drank 100 to 110 ml. between midnight and 1:30 A.M. and often had a second large drink in the early morning hours. The drinking of large volumes of water during the night

is of particular interest, as it shows clearly that the monkey did not sleep uninterruptedly throughout the night and did not void when it awakened enough to drink. In general, no correlation could be found between the patterns of micturition and water intake. In one monkey (MR 524) there were at least 12 hours between the last drink in any one day and the next voiding (FIGURE 4).

Since the monkeys were not weighed daily and no calculations were made of

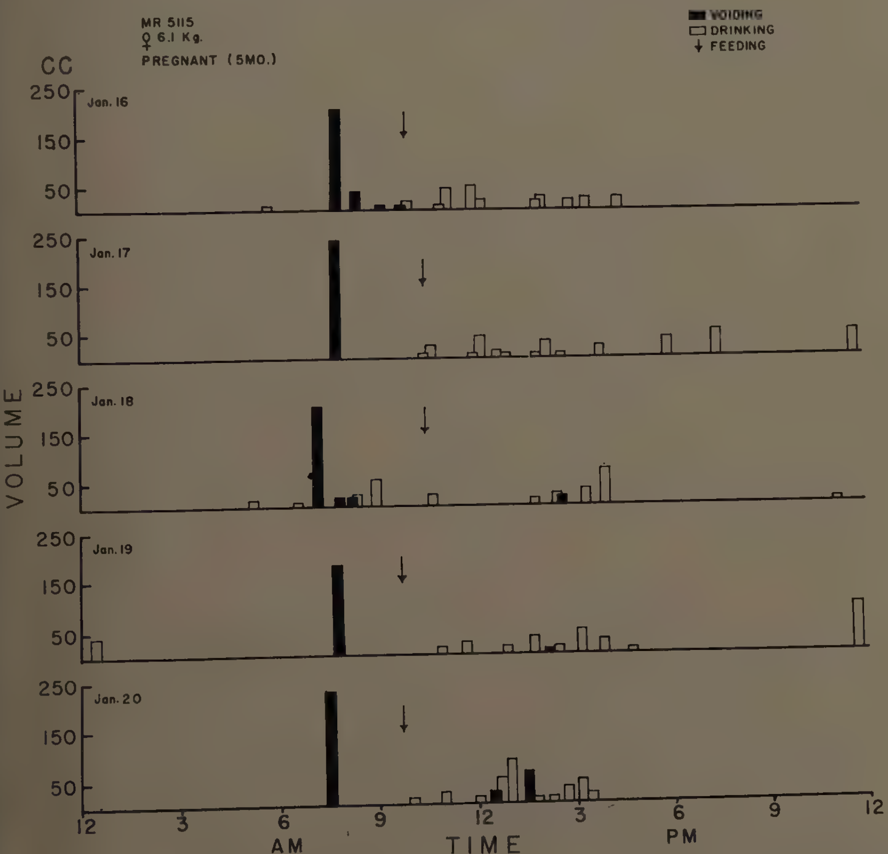

FIGURE 5. Patterns of voiding and drinking for 5 consecutive days in a pregnant monkey (MR 5115, 6.1 kg.). The arrow indicates the time when food was put in the cage and the chart changed.

insensible water loss or water content of feces, it is not possible to determine water balance from our data. Analyses of random samples of the food pellets in this study showed a water content of 8 to 10 per cent. Since the monkeys usually ate 100 to 200 gm. of food pellets per day, the amount of water received in their daily food intake amounted to only 10 to 20 ml. The small amount of water in the diet and the water of oxidation were not included in the figures for water intake (TABLE 1). This was mostly balanced out by evaporation from the drinking cup amounting to 10 to 15 ml. per 24 hours. Variations in water

intake or in urinary output could not be correlated consistently with phases of the menstrual cycle. It was not possible from our observations to account for an isolated daily water intake that was significantly higher or lower than the intake of other days.

For comparison, water intake was calculated on the basis of body surface area utilizing the formula of Lee and Fox (1933), who measured accurately the surface area in 6 rhesus monkeys of different ages and from this data derived a modification of the Meeh-Rubner formula where $S = 11.7W\ 2/3$, in which surface area (S) is expressed in square centimeters and body weight (W) in grams. The calculated mean daily water intake for each of the 20 monkeys studied ranged from 575 ml. to 2300 ml./sq. m., with a mean value of 1179 ml. The mean water intake calculated on the basis of weight was 81.3 ml./kg. The coefficient of variation for water intake calculated on the basis of body weight was found to be 37.3 per cent, as compared with a coefficient of 34.8 per cent for intake calculated on the basis of surface area. This indicates not only that there is great variation in water intake between individual monkeys, but that this variation is not solely dependent either on body weight or on surface area.

Adolph (1933) calculated the water requirements of animals utilizing both the surface area of the body and the energy requirement. He suggested that 1 cu. cm. of water per calorie of food intake was "roughly a convenient liberal standard of total water intake for any mammal". The calculated caloric value of the food pellets used in our studies is approximately 4 cal./gm., based on a content of 25 per cent protein, 8 per cent fat, and 10 per cent water. On this basis the estimated water requirement of 1 cu. cm. of water per calorie would correspond with the measured mean water intake in only about one half of the monkeys studied.

Discussion

Water intake. Many different theories have been advanced to explain the factors controlling water regulation. Adolph (1943) concluded after reviewing the subject that no one theory could apply to all organisms and to all living units. He reasoned that probably there was no "ultimate determinant" of water content, short of the whole organism and its environment, as anything less represented only a partial account of the adjustments concerned.

Species variation in water intake is well illustrated by the investigations of Ross (1930), who showed that two species of deer mice (Peromyscus) raised in the laboratory on a constant diet had a 32 per cent difference in water intake, while subspecies drank approximately equal amounts of water. The differences in water consumption are much greater in widely divergent species studied under similar conditions. For example, in a recent investigation by Schmidt-Nielsen *et al.* (1957), carried out at the oasis Beni Abbes in the Algerian Sahara, the donkey was found to require more than twice as much water as the camel. Atkeson and Warren (1934) studied the patterns of drinking in cows by means of an automatic self-recording water meter connected to the drinking cups in the stalls and obtained records of the exact amount of water taken and the time of day when it was consumed. The daily total intake averaged 102.6 lb. for the dry period, 140.3 lb. for the medium milk-production period, and 231.4 lb. for the high production period. The percentage of water consumed during the

night constituted 20 per cent of the total water intake in the dry period, 35 per cent in the medium production period, and 39 per cent in the high milk-production period. These investigators correlated water intake with body weight since they found that, when the water in the milk was subtracted from the total water ingested, the remaining water per hundred pounds of body weight was the same (6.2 lb.) whether the cows were dry or producing, provided they had eaten the same amount of dry food.

Richter and Brailey (1929) studied the water intake in a group of rats from the age of 30 to 160 days. They found the intake to be larger in the males than in the females and to increase gradually with age. Young animals drank more per kilogram of body weight than adults. The water intake in rats could not be correlated with body weight, but correlated very closely with surface area, being 800 ml./sq. m. in all ages studied. In a subsequent paper, Richter (1938) calculated water ingestion of several species of mammals on the basis of data gathered from the literature. He concluded that the water intake of normal animals and man was a function of surface area rather than of body weight and hence was directly related to metabolism. The mean daily water intake for the 20 monkeys studied in our investigations varied from 575 ml. to 2300 ml./sq. m., with a mean value of 1179 ml. Because of large variations in the water intake calculated on the basis of body surface area, other factors such as age, activity, metabolic rate, renal function, and possible central nervous system effects must be considered.

The fact that central neural mechanisms, especially those in the hypothalamus, play a role in the determination of water intake is well established by experimental studies in which water ingestion is greatly increased after lesions involving the supraoptic-hypothalamic-hypophysial system. Recently Andersson and McCann (1955) reported that electric stimulation of the hypothalamus in goats caused the animal to drink large amounts of water as long as the stimulus was applied, ceasing 2 or 3 sec. after the excitation was discontinued. Andersson (1956) reviewed the work of other investigators who had formulated various explanations for the cause and origin of the sensation of thirst and stated that his own experiments indicated that the hypothalamus played an important role in the regulation of water intake, without showing whether or not the hypothalamic "drinking area" was essential for the development of an urge to drink.

The effect of food ingestion on patterns of drinking in animals has been studied by many investigators since Longet (1868) first emphasized the importance of postprandial thirst. Gregersen (1932) found in dogs that almost all the water was taken within 2 to 5 hours after feeding, regardless of the time food was given, and Kleitman (1927) showed that in starvation the total water intake in dogs was only one fifth to one third of the intake under normal conditions. A few monkeys in our study appeared to take all their water in a period of 3 to 5 hours after food was put into the cage (FIGURE 4), while others regularly took a large drink during the night (FIGURE 6). The possible correlation between the patterns of drinking and food ingestion is difficult to demonstrate in monkeys because of their irregular habits of feeding and their ability to store as much as 200 gm. of food in the cheek pouches for later consumption.

Towbin (1955) studied thirst and hunger behavior in normal dogs as a basis

for his observations on the effects of vagotomy and sympathectomy. He reported that a normal dog varies the number of drinks rather than the size of each drink in response to changes in environmental temperature, and he interpreted this as indicating that the animal "allows itself to reach only a given degree of water deficit before drinking." He found that every adult dog favored

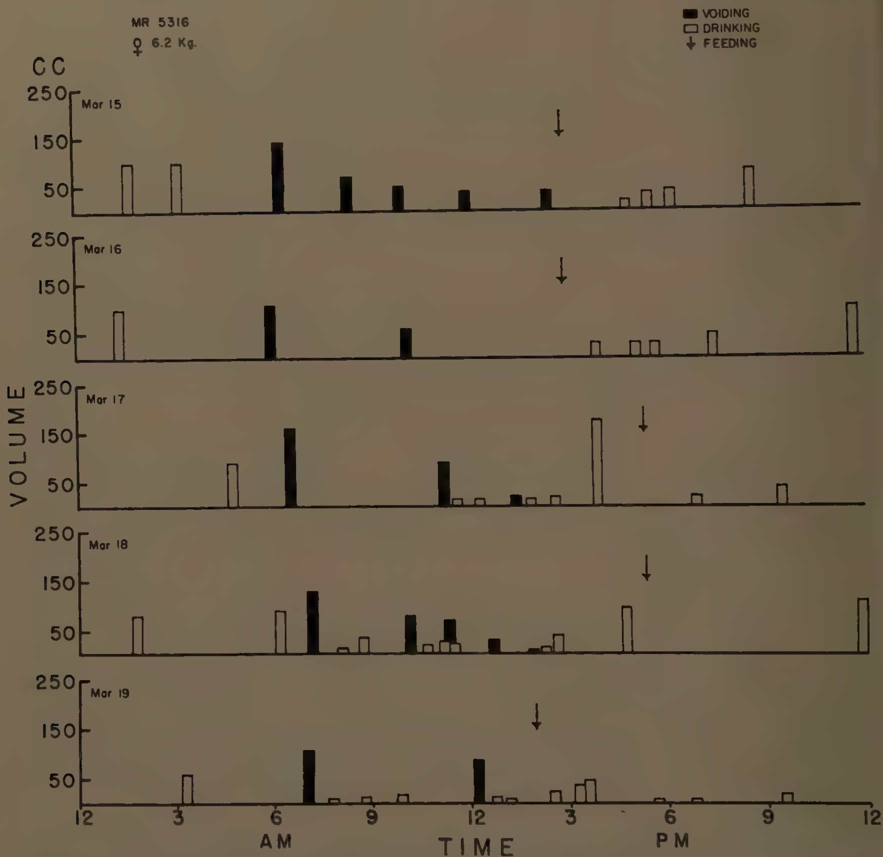

FIGURE 6. Patterns of drinking and voiding for 5 consecutive days in an adult female (MR 5316, 6.2 kg.). Large drinks of water around midnight and in the early morning hours were characteristic of this monkey. Arrow indicates the time at which food was put in the cage.

a characteristic size of drink that was not affected by the total food or water intake or by small changes in body weight. Contrary to Towbin's observations on dogs, most of the monkeys in our investigations did not favor a certain size of drink, but showed definite variations in the volume of individual drinks in any one day, the amount varying in some monkeys from 10 to 180 ml. In addition to the variations in the size of the drinks, patterns of drinking displayed considerable variations from monkey to monkey in contrast to the remarkable similarity in the patterns of micturition.

Micturition. This investigation shows that monkeys have a diurnal pattern of micturition. This adds another to the known diurnal rhythms related to the excretory system, since it has already been demonstrated by others that there are diurnal variations in water and electrolyte excretion in man that appear to be regulated by some neurological mechanisms involving the hypothalamo-hypophysial system.

Keith (1953) reviewed the findings of different investigators who had demonstrated that changes in the normal sleep rhythm, starvation, water and salt deprivation, and the administration of pituitrin, corticotropin, or desoxycorticosterone did not affect the diurnal excretory rhythm in man, and that short periods of hyperventilation reproduced the alkalosis and some of the excretory changes that occur spontaneously after sleep. Mills (1951) found that, despite the suspension of the 24-hour cycle of habits for 6 days, including inversion of the sleep rhythm, his subjects showed variations in temperature and urine flow on a 24-hour cycle, the variations being lower around midnight than around midday, and he suggested that the diurnal pattern was a manifestation of a hypothalamic rhythm, autochthonous for a period of time, but ultimately derived from external rhythms. Rosenbaum *et al.* (1952) studied the influence of cortisone administration on diurnal variation of renal excretion of water and electrolytes and suggested the possibility that the observed alterations were produced by "neurological action," since there was no evidence for direct action on the renal excretory mechanism.

Lewis *et al.* (1956) reported on the patterns of excretion of sodium and potassium in 8 human subjects living on an abnormal time scale of a 22-hour day for as long as 7 weeks, and they found clear evidence of the existence of an intrinsic 24-hour rhythm in several subjects. They pointed out that the reasons for the observed daily variations in electrolyte excretion are not yet known and suggested that, if a central nervous system mechanism were involved in the determination of the diurnal cycle of electrolyte excretion, it would be reasonable to suppose that it would have its origin in the hypothalamus-posterior pituitary complex. These investigators also suggested that animal experiments might yield valuable information on diurnal excretory rhythms because psychological influences could be removed to a greater extent than possible with human subjects. The long nonvoiding periods observed in the monkeys studied by us would obscure any possible diurnal variation in urine flow or electrolyte excretion and would make the collecting of fractional samples of urine impossible without disturbing the animal. Any procedure that involves handling the animal, such as catheterization or venipuncture, must be regarded as stress that can affect renal and bladder function.

A search of the literature has not revealed any reports on the patterns of micturition in primates. It was found in this study that both male and female monkeys voided a large amount in the early morning. The mean volume of the first morning voiding in 20 monkeys was 150 ml., but volumes exceeding 300 ml. were recorded in several instances. Some of the animals voided only once a day, and the mean nonvoiding period was 17.8 hours. The mechanisms that inhibit the contraction of the bladder in the normal monkey when it contains 200 ml. or more are not known at the present time. It may be that awakening in some way sensitizes the response to bladder distention, since monkeys are

found to void early in the morning at a time that can be correlated with marked increase in activity. However, several of the animals regularly awakened sufficiently to drink large amounts of water during the night without voiding at that time (FIGURE 6). The fact that the bladder in the monkey is not a true pelvic organ and hence rises into the abdomen as it becomes moderately distended may be a factor that facilitates its adaptation to large volumes. Cystometric studies in a number of unanesthetized monkeys show that volumes of 90 to 120 ml. can be introduced into the bladder before a contraction occurs.

There is an obvious similarity between the patterns of micturition in monkeys as revealed by our observations and the well-known fact that man does not usually void during the night and normally has the largest voiding on arising. The steps by which the infant monkey or child arrive at the adult diurnal pattern of micturition constitute another phase of this problem.

The logical assumption that the cerebral cortex plays an important role in regulating bladder activity is supported by the finding that alterations in the pattern of micturition in monkeys result from localized cortical extirpations, as presented in a preliminary report by Smith and Feldmahn (1954).

Summary

Patterns of drinking and voiding were investigated in 20 mature monkeys (*Macaca mulatta*) ranging in weight from 3.1 to 9.6 kg. by means of specially designed apparatus that automatically recorded on a time chart water intake and urine output.

It was found that monkeys, both male and female, exhibit a diurnal pattern of micturition characterized by a large voiding early in the morning with one or more smaller voidings during the day, and long nonvoiding periods, averaging 17.8 hours. The patterns of drinking showed greater variations from monkey to monkey and for the same animal from day to day than those of micturition. No correlation was found between the time of water ingestion and the time of voiding.

Results of investigations now in progress indicate that the cerebral cortex plays an important role in the regulation of the diurnal pattern of micturition.

Acknowledgments

The recording apparatus used in these experiments was obtained from the Taylor Instrument Company of Rochester, N.Y. We are grateful to Leslie Van Huben of this company for his generous assistance in adapting the equipment for our use.

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PARASITISM IN MONKEYS

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After sporadic exposure to the numerous species of parasites that may be found in the monkey—or perhaps one should say monkeys in recognition of the diverse species of these animals and their wide distribution—it is something of a surprise to be led to what at first may seem an impossible conclusion: that parasitism—principally helminthic—is not a major biological hazard for the monkey. It is not a problem, notwithstanding numerous instances that may be cited to the contrary for, be it remembered, the extent to which contact with man is fully recorded in a "cause and effect" relationship is by no means clearly registered in much of the literature dealing with the subject.

In so far as some of the parasites are concerned, the parasitoses of the monkey should be viewed in the same perspective as should domesticated and semidomesticated animals influenced by human husbandry. In their natural environment it seems likely that helminthic infections are minor ailments for monkeys when compared to the predations of carnivores, raptorial birds, and reptiles. That the life of the monkey is replete with these contacts is attested to by the frequency with which it harbors the intermediate stages of parasites that reach maturity only in these predators, to whose diet the monkey is a staple. The fact that some of the parasites lean on these essential avenues of biological transfer testifies to the normality and antiquity of the relationship. The monkey lends itself to parasitism with the greatest of ease and the species survives in considerable numbers despite the losses to be expected from these established facts. Epizootics with viral agents represent parasitism at its worst for the primate hosts. Enteric infections other than helminthic or viral may be far more significant causes of death than is generally appreciated. The very obviousness of the helminth parasites and often the pathology they induce is a clear invitation to credit which, in fact, they do not deserve.

Among the helminths, the nematodes are the parasites most frequently found; in numbers of species involved they are the dominant group, and among the nematodes found in monkeys two genera of worms stand out prominently for frequency of infection and parasitic importance. These genera are *Oesophagostomum* and *Strongyloides*. The members of the latter genus, represented by a number of species inhabiting, though perhaps not exclusively, a number of different species of monkeys, are probably the most ubiquitous of the intestinal nematodes. They are overshadowed in importance by worms of the former genus, *Oesophagostomum*. Concerning the eight or more species of esophagostomes found in primates it is not possible, in the absence of an extended morphologic study of specimens, to be certain of their taxonomic status. It seems probable that some of the specific names are synonyms. Concerning the number of species, it should be noted that swine in the United States alone harbor four species of *Oesophagostomum*. Likewise, it is not clear how much host overlap there may be among species of these monkey nodular worms. The pathology they induce in the intestinal tract of the primate hosts and the fre-

quency with which they are found are indicative of their importance. For example, *O. bifurcum* was the commonest parasite in a survey of 100 rhesus monkeys, reported from Poland by Bezubik and Furmaga (1959). This worm was present in 55 per cent of the animals; the next most frequent parasites were the whipworm, *Trichuris trichiura* (27 per cent), the spirurid *Streptopharagus pigmentatus* (24 per cent), and the hookwormlike *Ternidens deminutus* (21 per cent). If it may be assumed that the primate species of nodular worms develop in the same manner as do those species of esophagostomes found in swine, sheep, goats, and cattle, then it must be concluded that the pathology (nodule formation) in monkeys is a product of long exposure to infective larvae. In the case of the sheep nodular worm, *O. columbianum*, the large nodules do not develop as a result of primary exposure, but as a consequence of continued exposure. The initial infection period is one of sensitization, and the pronounced host reaction with nodule formation is a product of a sensitized and partially immune animal. The infective esophagostome larvae (an ensheathed third-stage worm) must pass the period of its development in this third stage, after exsheathing, embedded in the wall of the intestinal mucosa, where it normally passes through its third ecdysis to become a fourth-stage or preadult worm. This preadult worm is usually found free in the lumen of the intestine or colon or, if the host reaction is prompt and worm development slow, it may be trapped and retained inside the developing nodule. The fourth-stage larvae ultimately pass through a final molt to reach the fifth or adult stage. Normally, the adults inhabit the colon of the host, where they do not appear to be serious pathogens. They do not attach to the wall of the colon, do not suck blood, and do not contribute obviously to the pathology observed in the host.

In the absence of continuing exposure to infective larvae, the nodules from which newly molted fourth-stage larvae manage to escape must be subject to normal processes of tissue repair and ultimate fibrosis unless secondary infection exacerbates the infection at this point. Nodules from which the fourth-stage larvae do not succeed in escaping become enlarged and are a continuing target for the host repair processes. The ultimate fate of such an entrapped larva is death; for such a nodule, caseation, fibrosis, and calcification. Such nodules are monuments to host success in coping with an invading parasite. Thus, the host pays a price for its biological ability to wall off and destroy the nodular worm larvae. The larvae that escape the boundaries of the intestinal wall and wander in the peritoneal cavity are rapidly encapsulated in the omentum or mesentery. The tiny nodules, with fibrosed walls, hold small fourth-stage larvae. It seems improbable that such larvae ever escape to re-enter the lumen of the digestive tract and resume their normal life. Even in death the larvae are irritants. While the soft tissues of the worm are readily broken down and phagocytized, the external cuticle is highly resistant to enzymatic dissolution and remains as an irritating foreign body.

It is assumed that the mode of entry for the primate esophagostome larvae is by way of the mouth. It seems probable that this is true in large measure if comparison with the ruminant species is a valid one. That it may not be an exclusive mode of entry should be considered. Mayhew (1939) has shown in apparently well-controlled experiments with *O. radiatum* in calves that dermatitis and erythema developed at the site of application of the larvae and

that after the proper time interval esophagostome eggs appeared in the feces of exposed calves. There is no reason to believe that primate esophagostome larvae may not enter their host as successfully by this route.

In view of the rapidity with which esophagostome larvae reach the infective stage—a matter of days—and their ability to survive in this state for comparatively long periods of time, the problems of host density, fecal contamination, and area sanitation are introduced as significant variables in so far as the captive primate is concerned. While it cannot be stated that oesophagostomiasis is not of consequence to primates in their native environments, it appears certain that, as seen in captive primates, it is "created" by their environmental management, which of course can be highly variable.

In terms of sheer numbers of worms and the high incidence of infection in the host species of primates, the various species of nematodes in the genus *Strongyloides* are without equal. The life cycle is adapted to rapid development both inside and outside the host, and the infective (unsheathed) third-stage, or filariform larvae (as they are commonly called) are efficient skin penetrators. Many, but apparently not all, of the larvae that penetrate the skin make the vascular-pulmonary transit and arrive ultimately in the digestive tract, where they migrate, quite out of sight, embedded in the mucosal villi. All of these parasitic worms are females and, contrary to certain extant opinion, they produce eggs that develop parthenogenetically. The developmental phase in the host intestine is rapid. In the case of well-studied species, they may reach reproductive maturity in as little as four days (*S. ratti*). Prepatent periods ranging from eleven to eighteen days have been reported by Faust *et al.* (1934) for *S. stercoralis*, the human species experimentally introduced into the dog. *S. papillosus* of sheep regularly has a prepatent period of ten days when introduced into the domestic rabbit. Following skin exposure of lambs, Turner (1955) reported the first appearance of eggs of *S. papillosus* to be at nine days. Infections consisting of several thousands of worms (*S. papillosus* in rabbits) are not macroscopically obvious in the small intestine of sacrificed animals, nor could one presume to call such a heavily parasitized gut abnormal. A portion of a cross section from the small intestine of a parasitized rhesus monkey (FIGURE 1) shows little evidence of serious damage. Of necessity, there is some local destruction of cellular elements of the mucosa, but there is no evidence of gross intestinal damage. In this particular cross section of the intestine there were no fewer than six sections of parasites or nests of eggs. It seems not unreasonable to estimate that such a sample represents an infection that would be classified as "heavy" for the whole of the small intestine. The abundance of embryonated eggs in the feces of such an infected animal might readily suggest that the animal was "suffering" from a "severe" strongyloidiasis.

The hazard in a *Strongyloides* infection for the captive primate resides in the rapidity with which the embryonated eggs can reach the third larval, or infective, stage. This period may be as little as 2 days. An additional complication of the *Strongyloides* life cycle is the fact that, particularly in the monkey-infecting species of the genus, the free-living bisexual generation which characterizes this genus of nematodes operates to magnify the infective larvae manyfold. Thus within as few as 5 days 1000 eggs of the parasite escaping from the host in the feces may be transformed into as many as 20,000 infective

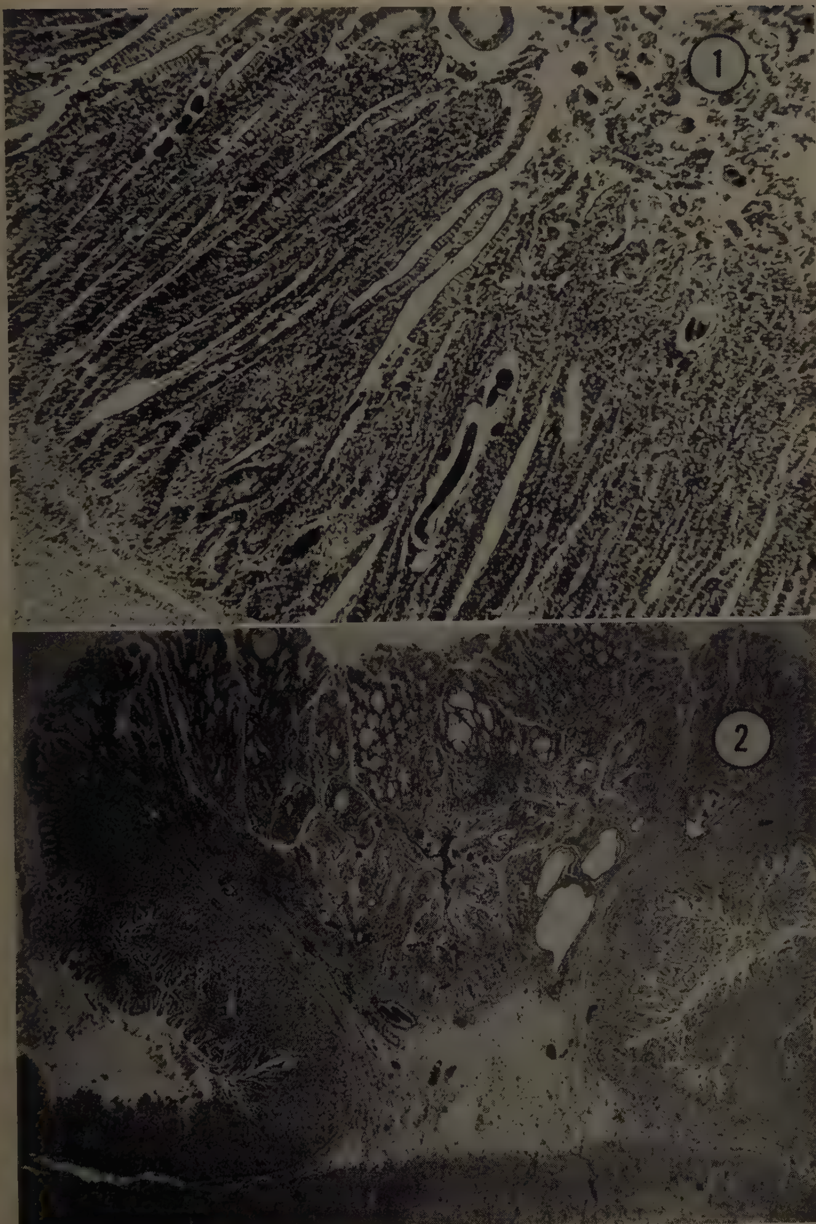


FIGURE 1. Histological section from the small intestine of a rhesus monkey showing a section of a parasitic female of *Strongyloides*, possibly *S. simiae*. A string of three eggs can be seen (*upper left*) and a single embryonated egg (*right center*).

FIGURE 2. A small "tumor" induced by the trichostrongylid nematode *Nochtia nochti* in the stomach of a rhesus monkey.

larvae by reproduction of the free-living generation. Such a reproductive boost for the parasite constitutes a danger for the susceptible host that must live in close proximity to this helminthic population explosion. Massive, often disastrous, enteric infection may occur; coupled with other intestinal infections, the consequences can be grave.

When *Strongyloides* infections in primates are a possible problem, recently developed and apparently effective antihelminthics may be used. Although I know of no use of them for primates other than man at the present time, dithiazinine iodide* and pyrrovinyquinium chloride,† which have been used with much success against *Strongyloides* in man, offer interesting possibilities. The former has been used successfully against the dog parasite, *S. canis*, which is often a serious pathogen, especially in puppies.

A number of spirurid nematodes, all of which require an arthropod intermediate host, are found with some regularity in both rhesus and cynomolgus monkeys; among those more commonly encountered are *Streptopharagus armatus*, *S. pigmentatus*, *Physaloptera caucasica* and *Gongylonema* sp. The first three worms, inhabiting the stomach, are obvious parasites; *Gongylonema*, inhabiting the musculature of the esophagus and causing no major pathology, is relatively benign and apparently is observed seldom. Various dung and meal beetles, cockroaches, and crickets have been implicated as intermediate hosts for some of the species in the genera *Physaloptera* and *Gongylonema*.

A small trichostrongylid nematode, parasitic in the stomach of the cynomolgus monkey, is an interesting example of parasitic specialization. These small red nematodes, in comparatively small numbers, are able to induce a specific pathological response that obviously favors their existence. The so-called tumors induced are located in the comparatively narrow zone between the fundic and pyloric stomachs. Such a "tumor" is shown in FIGURE 2. Sections of the parasite, *Nochtia nochti*, may be seen deep in cavities in the submucosa. Eggs, laid in long beadlike chains, slowly erode their way to the lumen of the stomach. Details concerning the morphology of the parasite and its biology, together with the first report of the role of this nematode in tumor development in the stomach of monkeys, were presented by Bonne and Sandground (1939).

Cross sections of these small worms examined microscopically under oil immersion reveal the longitudinal ridges of the cuticle. There are eight, longitudinally disposed on the upper half of the worm's cuticle, and an equal number on the ventral surface. Laterally, a comparatively broad alar thickening is observed that is concavely channeled. The worm resembles a reaming tool which, in fact, it really is.

The inflammatory reaction induced by the parasite is marked, being characterized by a polymorphonuclear reaction in the mucosal element of the tumor, where direct contact with worms occurs, and by an eosinophilic and plasma-cell infiltration in the deeper submucosal portions. The constancy of the reaction and the specificity of its induction at a precise anatomic location emphasize an insufficiently appreciated character of trichostrongylid nematodes in general, namely, their exacting requirements concerning location in the upper digestive tract. *Trichostrongylus axei*, a common parasite of the stomach of equines and

* Delvex, Eli Lilly & Company, Indianapolis, Ind.
† Vanquin, Parke, Davis & Company, Detroit, Mich.

the abomasum of ruminants obviously prefers a region of very low *p*H values; others, such as *T. colubriformis*, may be found in the anterior portion of the small intestine as well, while still others, such as *Nippostrongylus* in the rat, is consistently a parasite of the small intestine. Apparently, *Nochtia nochti* is a specialist among specialists. Only one other example of such specifically induced pathology is known to me. *Braunina cordiformis*, a strigeoid trematode parasite in the stomach of the porpoise, specifically induces the submucosa to grow outward and completely surround the bulbous hind body of the worm, while the forebody wraps itself around the thin, highly vascularized layer of host tissue—a veritable "sandwich": host between parasite! The parasite and the enveloping host tissue are shown in FIGURE 3. It would not be expected that such an elaborate mechanism had been evolved for the simple purpose of attachment, yet it does serve that function.

Another group of nematode parasites that appears to be more or less common in various species of monkeys is the metastrongylid lungworms of the genera *Filaroides* (= *Oslerus*) and *Metathelazia*. The importance of these parasites is debatable, but there is little doubt that in large numbers the pathology they produce is well defined. FIGURE 4 depicts an atelectatic area in the lung of a squirrel monkey, *Saimiri sciurea*, harboring a heavy infection with *F. barretoi*. The normal architecture of the lung is largely destroyed by the movement of the worms, but vascularization remains essentially intact, except for small hemorrhagic foci that are not numerous. Such areas are infiltrated heavily with round cells, and some giant cells are seen. Polymorphonuclear leukocytes are not abundant, and the pathological picture is not that of a pneumonitis. The particular area photographed for FIGURE 4 was selected to show various stages of embryogenesis in a female worm. The embryos are active, and many cross sections as well as oblique sections of free larvae could be seen in the infiltrated area surrounding many of the adult worms. They escape from the lung via the bronchi and trachea and are discharged by the host either in the sputum or by being swallowed and passing out in the feces. They might be taken in the latter instance for *Strongyloides* larvae. Many horizons show sections of adult worms in apparently normal bronchioles with little evidence of the heavy inflammatory exudate that characterizes infections with *Metastrongylus* in swine and *Dictyocaulus* in sheep and cattle. It is problematical whether a drug such as cyanacethydrazide,* which is effective to some degree against *Dictyocaulus*, could be employed against these monkey lungworms.

The whipworm *Trichuris* is found in both the rhesus and cynomolgus monkey with modest frequency, but never in such numbers as to be of real consequence as a pathogen. There is evidence that at least one species of *Trichuris* other than *T. trichiura* occurs in the rhesus monkey (Bezubik and Furmaga, 1959). Of more importance when it does occur is *Capillaria hepatica*, normally a parasite of the rat, but able to develop well enough in both the monkey and man. The parasite migrates through the liver tissue and lays numerous eggs that accumulate and are released after the death of the host. The eggs may be released and spread by passage through a carnivore. I have observed also,

* Dictycide, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

FIGURE 3. The trematode *Braunina cordiformis* in the stomach of the porpoise, showing the outgrowth of the submucosa surrounding the hind body of the worm and the enveloping forebody of the parasite. Not shown is the long slender posterior tip of hind body that penetrates deeply into the submucosa.

FIGURE 4. Sections of lungworm *Filaroides barretoi* in lung of squirrel monkey. Developing eggs are seen (*upper right*). Numerous embryos are packed in the uterus.

in the liver of infected wild rats, that blowfly maggots are superb agents for quickly releasing the eggs from the liver substance and mixing them in soil where embryonation can take place. In either case, embryonation must occur outside the body of the host. The predator does not become infected directly by ingesting, for example, a rat liver containing *C. hepatica* eggs. Transit through the gut of such a predator does no damage whatever to such eggs.

Oxyurids are not encountered often. When infections with *Enterobius anthropopitheci* do occur, the minute worms are rather numerous, suggesting that the well-documented method of transfer seen with the human pinworm, *E. vermicularis*, namely, "seat-picking," is probably a part of man's pre-Hominian heritage. A species of oxyurid, *Oxyuronema atelophora* (Kreis, 1932) from the spider monkey, has been observed by the writer on a single occasion. The abundant worms, in all stages of development, came from a "pet" monkey that had been under treatment for an "intestinal disturbance." Kreis was of the opinion that the worms were pathogenic. The esophageal "spears," three in number, which he depicted and interpreted as the "instruments" for lacerating the colonic mucosa, can be viewed less imaginatively today as the cuticular lining of the three esophageal radii, quite incapable of functioning as lancets. Nothing is known about the bionomics of this worm other than the fact that when it is present it may be in large numbers in the colon and rectum.

Certain pseudoparasitic nematodes should be distinguished from true parasites. The worms found by Sandground (1939) in the stomachs of a number of Javanese monkeys and described by him as *Cephalobus parasiticus* were thought by Goodey (1939) to be *Turbatrix rediviva*, a free-living rhabditid nematode found abundantly in paper hanger's sour paste. Similarly, other contaminant rhabditids can be present. One of the two worms described in 1933 by Kreis and Faust as *Rhabditis macrocerca* and observed by them as contaminants on dogs and on two species of monkeys, in their animal quarters in New Orleans, I have observed in recent years as a contaminant of the soiled body hair of diarrheic rabbits in New Jersey and, recently, as a contaminant in the feces of captive nutria in Maryland. Apparently this worm is a widely distributed organism with a predilection for animal excreta. It should be noted that this nematode has the morphological features that characterize the genus *Rhabditella* (Cobb, 1929; Chitwood, 1933). *Rhabditis macrocerca* was referred to the genus *Rhabditella* by Steiner (1943). The correct taxonomic designation thus becomes *Rhabditella macrocerca* (Kreis and Faust, 1933; Steiner, 1943). Such free-living saprophagic nematodes might be confused by the uninformed with the bisexual generation of *Strongyloides*.

Certain nematodes have been omitted from this discussion of monkey parasites for a variety of reasons. In a few instances omission has been due to my belief that certain parasites are of no more than casual interest. Furthermore, no attempt is made to assemble here a complete or even nearly complete catalogue of monkey parasites. There are other nematodes evidence for which is equivocal; the parasite well known and well publicized that has never turned up in the numerous collections submitted to me for identification. Such a parasite is *Ternidens deminutus*. There is substantial evidence that it is a widespread parasite of man in some areas of Africa, where the monkey manages to spread it, and hygiene or deficiencies thereof favor its survival in human

populations (Sandground, 1931). On the other hand, there are disquieting statistics, such as those of Habermann and Williams (1957), indicating a very low incidence in 615 rhesus monkeys that they examined. To counter this one must weigh the incidence of *T. deminutus* reported by Bezubik and Furmaga (1959) in 100 *Macaca mulatta* in Poland (21 per cent). On the basis of the excellent photomicrographs of the anterior and of specimens of *T. deminutus*, published by Amberson and Schwarz (1952), I suggest that the worm is a much more serious parasite as an adult than are the members of the genus *Oesophagostomum*. The heavy-walled and globose stoma with the basal teeth is, like that of *Strongylus vulgaris*, *S. equinus*, and numerous hookworm species, admirably equipped to attach to the mucosa for the purpose of sucking blood. It is not likely that such good equipment goes unused by the parasite.

Finally, in coming to that large and poorly appreciated group of monkey nematodes, the filarids, one is tempted to acknowledge their prevalence in monkeys as subcutaneous, cavity, or connective tissue parasites, and to abandon them to the appropriate specialists. It is pertinent, however, to indicate how very difficult it is to accumulate precise and adequate information about the several genera and numerous species. Most survey work has been based on the incidence and morphology of the various microfilariae in the blood. Undoubtedly, the most extensive surveys have been those conducted by Clark (1931) and McCoy (1936) dealing with the filarid parasites in Panamanian monkeys. When the adults are located in the peritoneal or pleural cavities or in the heart, there is a reasonable chance that they will be found, often in fairly large numbers. However, it is common for adult worms to be found only with difficulty. For modest-sized or small filarids located in connective tissue, the search for parasites may well be a major undertaking. It is seldom that a parasite such as the undescribed species of *Dirofilaria* reported by Price (1959) will reveal its presence externally. The parasite in question could be visualized beneath the skin in the inguinal region of *M. irus*. The undescribed species was accompanied by another species, *D. magnilarvatum*, which was found in the deeper subcutaneous tissues and described as a new species. Filarial parasites of monkeys have been reviewed recently by Webber (1955).

With the present-day international traffic in monkeys, no one can be certain of the possible sequelae of bringing such filarid-infected monkeys into new territory. The risk is probably small, but is still definite.

The Monkey as Host for Cestodes and Trematodes

When one surveys the rather extended list of larval tapeworms reported to exist in various primates, the conviction grows that these animals are extraordinarily hospitable to these parasites. Thus, in addition to serving as hosts for the wide-ranging hydatid cysts of *Echinococcus*, primates, including man, and a number of other mammals have been reported to harbor assorted species of coenuri and cysticerci, which have been allocated to appropriate genera and species. It has always seemed to me grossly out of place to associate the coenurus of *Multiceps serialis*, from the brain of a monkey and from subcutaneous cysts on baboons, with a parasite whose normal range of hosts is best exemplified by the jack rabbit of the Great Plains region and the dog and coyote.

Occasionally larval cestodes of a distinctive type, allocated to the genus *Tetrathyridium*, have been reported from the monkey. These tetrathyridia, assumed to be larval stages of tapeworms of the genus *Mesocestoides*, which develop as adults in the intestine of various carnivores, are best considered as analogous to the cysticercus, although without the fluid-filled bladder. I have examined several dozen of these unusual proglottid-shaped larvae, which were found free in the omentum and mesenteries of a rhesus monkey. That they develop there cannot be doubted; that the monkey is not entirely passive in the matter also is obvious for, if these tetrathyridia can be entrapped by host tissue, a thick fibrous wall can be laid down around them, as shown in FIGURE 5. This cyst was one of a cluster attached to the mesentery. In the section, the invaginated and unarmed scolex can be seen as a recessed appendage in the solid, muscular, proglottidlike hind body, which obviously is motile when the "worm" is free in the body cavity of the intermediate host.

Once they are entrapped these tetrathyridia can be destroyed by the host. The cyst wall is breached, and the immobilized parasite is engulfed in a mass of invading host cells. In FIGURE 6 such an harrassed scolex is shown, completely surrounded by a mass of inflammatory cells, chiefly polymorphonuclear leukocytes. The necklike connection with the hind body is broken and, although the organism is still viable and presumably infective, its position vis-à-vis the host is purely defensive.

That this siegelike "biological warfare" favors the host may be seen in FIGURE 7, which shows a cyst near those depicted in the two preceding figures; here the breakdown and destruction of the tetrathyridium is practically completed.

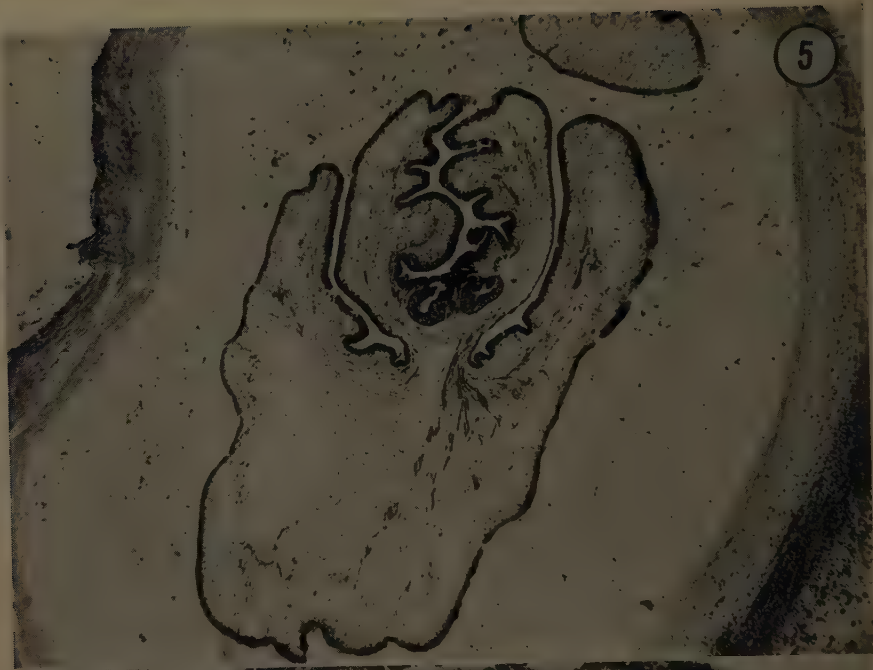
It seems likely that the encysted cestode larvae from a gibbon, *Hylobates lar leuciscus*, reported by Sambon (1924), are the same or a closely related species.

Still other taenoid cestode larvae are encountered in the monkey. I have histological sections of a mesenteric lymph node from a squirrel monkey that contains, in addition to numerous microfilariae, a single cysticercus of an indeterminate cestode. The only positive identifying feature of this exceptionally small larva is the fact that the scolex has an armed rostellum. None of the sections showed the hooks with clarity, so that measurements were impossible.

It is of interest that Bezubik and Furmaga (1959) found *Cysticercus tenuicollis*, a distinctive larva normally found in the mesenteries of sheep, in a single rhesus monkey among the 100 *M. mulatta* they examined. This cysticercus is the larva of *Taenia hydatigena*, a tapeworm of the dog.

Among the several tapeworms that have been reported from monkeys, I have seen only worms of the anoplocephaline genus, *Bertiella*, apparently *B. studeri*. These worms do not appear to occur in great numbers.

Concerning trematodes as parasites of monkeys, the role of these animals as experimental hosts for various species of schistosomes is too well known to merit more than passing mention. Of particular interest appear the studies of Hsü and Hsü (1956) concerning strain differences revealed in *Schistosoma japonicum* from various locations in the Orient, when these blood flukes were studied in monkeys from the different areas. Epidemiological studies had



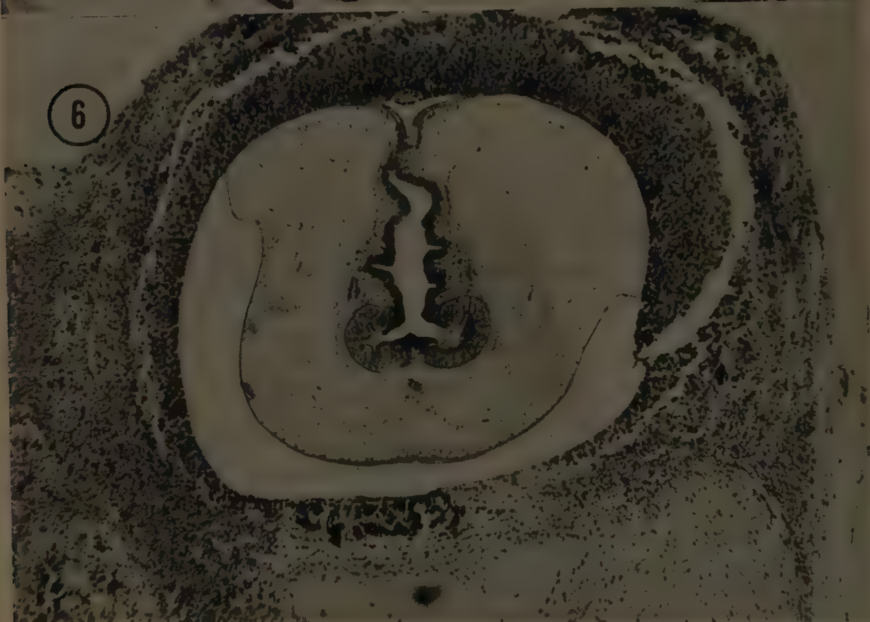


FIGURE 5. Tetrathyridium (larval cestode) encapsulated in a thick-walled fibrous cyst in the omentum of a rhesus monkey. The suckers on the invaginated scolex may be seen in the recessed anterior end of the organism.

FIGURE 6. The scolex of another encapsulated tetrathyridium that is in process of being destroyed by the host. The "head" portion of larva has been severed from the hind body, and the scolex is completely enveloped by an inflammatory exudate composed of polymorphonuclear leukocytes.

shown that *S. japonicum* did not develop in the human population of Formosa, although it was present abundantly as a zoophilic parasite. A brilliant series of experiments involving *M. cyclopis* of Formosa, *M. fuscatus* from Japan, and *M. philippinensis*, infected with *S. japonicum* from the three islands, revealed that "the Formosan monkey was highly susceptible to the Japanese and Philippine strains but was a relatively poor host for the Formosan strain." Comparatively, "Philippine and Japanese monkeys made reasonably good hosts for the Formosan strain of the parasite."

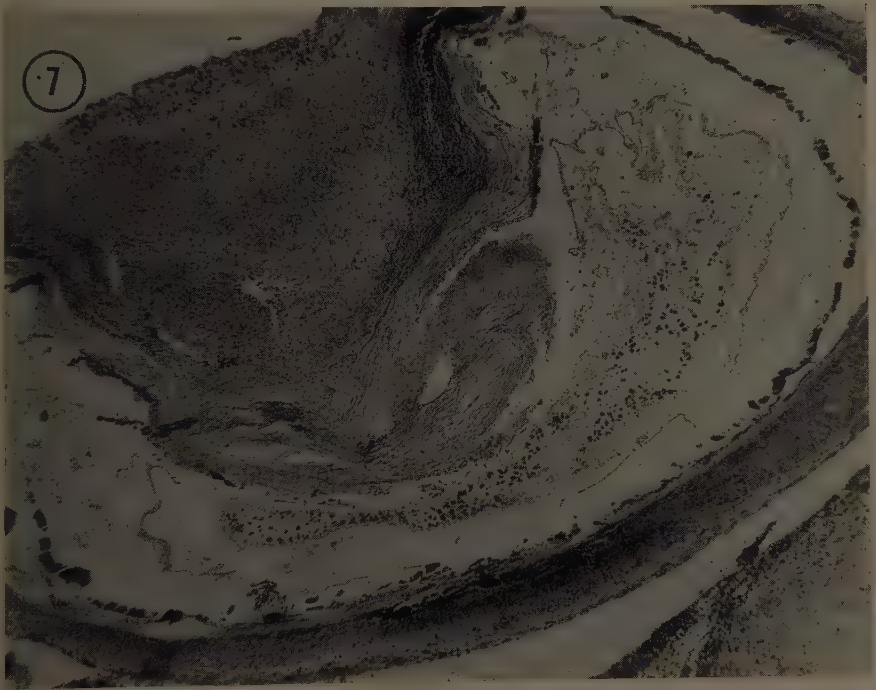


FIGURE 7. The remains of an encapsulated tetrathyridium that has been killed and almost completely destroyed by the host. It was adjacent to the encysted worms shown in FIGURES 5 and 6.

Notable too is the emerging picture of the "dog-face baboon" *Papio doguera* as an important reservoir host for *S. mansoni* in southeastern Kenya, East Africa (Miller, 1959*a*).

References in the literature to natural trematode infections in primates are not numerous. Worthy of at least passing notice and comment is the fact, seemingly extraordinary, that Bezubik and Furmaga (1959) found *Fasciola hepatica* in 5 per cent and *Ogmocotyle indica*, a notocotylid trematode parasite of sheep and cattle, in 3 per cent of the 100 *M. mulatta* examined. Both of these flukes are normal parasites of sheep and cattle. The occurrence of such parasites in the rhesus monkey at what seems to be somewhat more than a merely

accidental level emphasizes the remarkable susceptibility of these animals to a wide range of parasites. B. Bezubik has informed me (personal communication) that *O. indica* also parasitizes cattle.

Of the trematodes found in the monkey only *Watsonius watsoni* has been viewed as an indigenous monkey parasite. What seem to be rather heavy infections have been noted with this worm in a primate host. Pick and Deschiens (1947) reported infections with this fluke in baboons (*P. sphinx*) consisting of from 200 to 1000 worms in the lower digestive tract.

About two years ago, I received two living flukes in a tube of Eagle's tissue culture medium,* and assumed that the worms were *W. watsoni*. These worms were moving actively and were of an orange-red color. Examination with a dissecting microscope under reflected light showed a surface netlike pattern suggestive of the *système vasculaire superficiel* reported by Pick (1948) for *W. watsoni*. The worms remained active for several days when left at room temperature prior to formalin fixation. A more complete examination of the fixed specimens revealed that they could not be *W. watsoni*, and that they conformed on all points of morphology with *Gastrodiscoides hominis*. This worm has been found repeatedly in man and pigs in Assam and elsewhere in the Orient. It has also been found in the mouse deer in Malaya (Khalil, 1923) and in the common field rat in Java (Sandground, 1940).

A few months later, the large intestine and cecum of a freshly killed rhesus monkey containing several hundred small red flukes was placed at my disposal.† These worms, somewhat smaller than the two received earlier, were clearly *G. hominis*; they were apparently immature for scrapings of the mucosa, after removal of the worms failed to reveal eggs upon microscopic examination. Some of these worms were fixed with warm formol-saline under light pressure between glass slides. Following fixation, the red color disappeared and the worms turned a somewhat mottled, dirty-grey color. One of these lightly compressed flukes is shown in ventral view in FIGURE 8. The location of the genital cone shows clearly in this macrophotograph. One of the two larger worms fixed without compression is shown in FIGURE 9. The margins of the disk-shaped hind body are folded in and almost completely hide the acetabulum. High lights in the photograph make visualization of the genital cone difficult, although it was clearly seen in the specimen. Only a few references to this species of trematode as a parasite of monkeys have been found to date. Belding (1952) states in a textbook, without citing any authority for it, that it occurs in "monkeys." In a subsequent textbook Belding (1958) has omitted the reference without making other changes. The parasite had been reported earlier in a similar fashion by Hegner *et al.* (1929, 1938). Several additional occurrences of this fluke in monkeys have been observed and reported to me by Harry C. Fegley (personal communication, 1960). One other verbal report, almost certainly correct, of the occurrence of this worm has been received from another observer. Thus it must be concluded that the parasite is not really rare in monkeys from Asia although the usually authoritative catalogue of primate parasites by Stiles and Hassall (1929) makes no mention of it.

* Kindness of Dr. Allen W. Hayen, Wyeth Laboratories, Radnor, Pa.
† Kindness of Harry C. Fegley, Merck, Sharp & Dohme, West Point, Pa.

Acanthocephala

The thorny-headed worms are not abundant parasites of monkeys in terms of numbers of species but, for some of the smaller monkeys under conditions of confinement, they have the lethal property of accumulation to serious levels. *Prosthenorchis elegans* seems to be the worst offender on record. I have identified this parasite from material submitted from a squirrel monkey, dead of

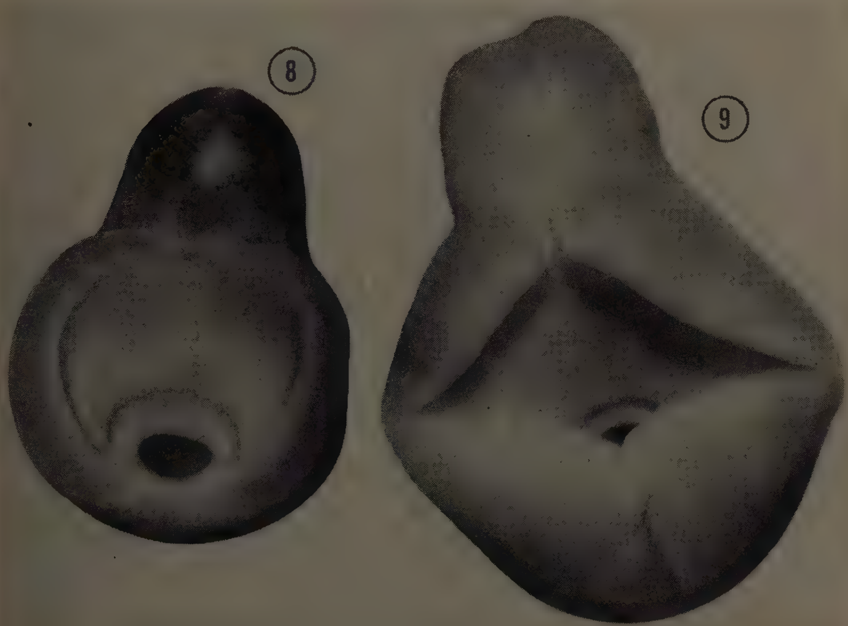


FIGURE 8. *Gastrodiscoides hominis*, an amphistome fluke from the cecum of a rhesus monkey, fixed under light pressure. The lateral margins of the cuplike hind body outline the ventral cavity that functions to magnify the adhesive ability of the muscular acetabulum. The light area on the forebody is the genital cone. This worm, somewhat larger than most of the worms of this infection, measures 4 × 5.5 mm. in maximal breadth and length.

FIGURE 9. A larger specimen of *Gastrodiscoides hominis* fixed without pressure. The lateral margins of the hind body are folded inward, and posteriorly almost hide the acetabulum, the anterior edge of which may be seen in the depths of the cavity. In lateral and dorsal views the hind body appears globose. The maximal dimensions of this worm (fixed) are 5.5 × 7 mm. The genital cone is scarcely visible because of high lights; actually it is quite prominent.

undetermined causes, and from two pet monkeys that died under similar conditions. In view of the experience of others (Takos and Thomas, 1958), this parasite must be viewed as a potentially serious pathogen where roaches (the intermediate host) have access to monkey feces and the monkeys in turn have access to roaches, a common occurrence.

Arthropoda

The nymph of the wormlike arthropod *Armillifer armillatus* is found with modest frequency encysted as a benign parasite in the viscera of the rhesus

and cynomolgus monkey. A cross section of such a nymph is shown in FIGURE 10. The nymph is enclosed in a fibrous cyst wall and seems, in the small numbers seen by myself, to cause no pathology. The adult pentastome parasite is an inhabitant of the lungs of pythons. Distinctive eggs of the adult worms are passed in the feces. They contain four-legged larvae known to infect both man and monkeys.

Lung mites of the genus *Pneumonyssus* seem to be universal parasites in the lungs of the rhesus monkey. Habermann and Williams (1957) indicate that they observed about 16 per cent infection with lung mites in 615 rhesus monkeys. Fremming *et al.* (1957) suggest an incidence of 100 per cent, a figure that I think is approximately correct, considering the remarkable reproductive and survival capabilities of mites in general. The lung mites in monkeys obviously cause some pathology, but it is difficult to believe that they can be major pathogens: they are too numerous and monkeys survive in too large numbers for such a conclusion to be acceptable. A lengthwise section of a mite in the lung of a rhesus monkey is shown in FIGURE 11. An egg is seen *in utero* and the genital opening is shown clearly on the ventral side of the mite. Habermann and Williams (1957) showed a section of monkey lung with lung mites (their Figure 6, p. 24) including a gravid *P. simicola* (*P. foxi* was placed as a synonym by Oudemans, 1935). The larval mite, still *in utero*, seems fully developed and the genital opening of the female can be clearly seen. These figures somehow make it difficult to understand the conclusion of Fremming *et al.* (1957) that the "life cycle of *P. foxi* remains an enigma." It is clear from the photomicrograph in the Habermann and Williams article that *P. simicola* females produce large larvae one at a time that are too large ever to be passed in the circulation. Thus transfer from host to host must be via the respiratory passages unless one is prepared to believe that they can burrow through muscular and connective tissue more or less easily. Fain (1959) has discussed monkey lung mites at length and has provided appropriate keys for the males and females of the ten species in the genus *Pneumonyssus* that he considered valid.

Protozoa

In general, the monkey has a spectrum of enteric protozoa that is essentially a replica of the pattern of protozoa seen in man. All of the evidence points to the probability of interchange where contact between man and monkey is established. Morphologic evidence alone might be suspect, but over the years the utilization of the monkey for experimental studies has permitted the conclusion that cross infection occurs.

The matter of pathogenicity and importance is so inextricably interwoven with other variables, such as diet and intercurrent infection, as well as crowding and sanitation, that the subject defies simple analysis or handling on a casual basis. It is easy, of course, to point out that amoebic dysentery and amoebic abscesses occur. The severity of *Entamoeba histolytica* infections may depend ultimately on strain differences of the parasite. How may we be sure that the manifestation of apparent health in infected monkeys is not really the aftermath or product of high infant mortality? Infant mortality is a subject about

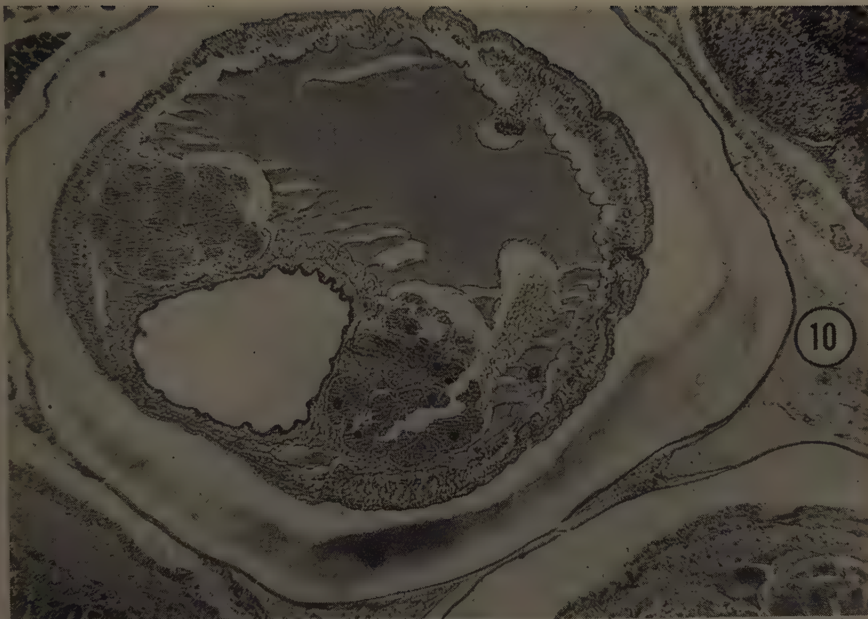


FIGURE 10. Cross section of *Armillifer armillatus* nymph encysted on the small intestine of a rhesus monkey (*lower left*). A lymph node is seen (*upper right*), and another *A. armillatus* nymph (*lower right*).

FIGURE 11. Lengthwise section of *Pneumonyssus simicola* in lung of rhesus monkey, including a section through leg I, showing musculature. Body musculature of all four legs on one side of body is visible. Single developing egg is located *in utero* posteriorly; genital pore may be seen near the anterior margin of the egg on ventral side of the mite. For a later developmental stage of this mite species progeny, see Figure 6, p. 24, Habermann and Williams, 1957.

which our ignorance is almost complete. It may be a highly important matter. It is suggested in Ruch's admirable handbook (1959) that *E. histolytica* is intrinsically less pathogenic for monkeys or that the monkey is more resistant than man.

While most of the intestinal protozoa of man and monkeys are very similar, there are a few spots where emerging differences may be noted. Thus the study of *Balantidium* in man, monkey, and pig may resolve the question of whether *B. coli* is the same in these hosts or whether the *Coli*-like *Balantidium* in monkeys is distinct. It should be noted that a spider monkey, *Ateles* sp., harbors a distinctive species, *B. wenrichi* (Krascheninnikow, 1960).

The subject of monkey malaria is so broad and involves so many species of both the plasmodia and monkeys that I am unwilling to do more than direct the interested reader to the serviceable list of references on the subject in Ruch (1959), which should suffice for ordinary purposes. The only comment that seems appropriate is that monkey malaria, in so far as the ordinary laboratory monkey is concerned, seems to be a negligible factor. Only a single reference will be offered in the area to call attention to the seemingly high incidence of infection with *Hepatocystis kochi* (75 per cent) observed by Miller (1959*b*) in a survey of 122 *P. doguera* baboons.

Summary

The subject of parasitism in monkeys is discussed briefly from a restricted point of view.

The thesis is offered that monkeys do not suffer from serious parasites under normal conditions and that, when they do so under conditions of captivity, the matters of hygiene, sanitation, nutrition, and crowding become increasingly important variables.

Certain of the nematode parasites are discussed with particular regard to their frequency and their potential pathogenicity.

The specific nature of the pathological responses that some parasitisms are able to elicit from their hosts is presented on the basis of two examples, one a tumor-inducing nematode in the stomachs of monkeys, the other a trematode parasite in the stomachs of porpoises.

The role of the monkey as an intermediate host for cestodes is presented briefly, and the defensive capability of the monkey against the parasite is pointed out.

A fluke parasite, *Gastrodiscoides hominis*, long known as a parasite of man and the pig in Asia as well as of a Malayan mouse deer and of Javanese rats, is reported as a parasite of monkeys, apparently for the first time.

The problem of enteric protozoa in the monkey is discussed briefly, and the similarity of the parasites to those in man is emphasized.

Acanthocephala and pentastomes as monkey parasites have been discussed briefly.

The ubiquity of lung mites of the genus *Pneumonyssus* as parasites of the monkey has been emphasized, the ovoviviparity of these mites pointed out, and the suggestion made that transfer from host to host must be by means of contamination via the respiratory passages.

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INFLUENCE OF CROWDING ON MONKEY HEALTH

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The requirements of commercial laboratories producing vaccines derived from infrahuman primate tissues involve the maintenance of large primate colonies. These large husbandry programs include rapid population turnover. Inventories may number fifteen hundred to three thousand animals. Under fluctuating production conditions, desirable inventory limits are often exceeded, resulting in crowding and mixing of animal groups. A tendency toward the grouping together of large numbers of monkeys has been coupled with these inventories. This practice of "gang caging" has had much appeal based on the utilization of floor space, low cage cost, and labor economy. These methods appear attractive when compared to the expense of isolation or small group husbandry. One is tempted, therefore, to extend the magnitude of large group maintenance to the fullest. Disease and social conflict, however, become major problems that invalidate the economies.

Gang-cage methods have raised the following question: "How many square feet of cage space are required per monkey?" If there is an answer to such a question, it should be stated in terms of a minimum, with no limit imposed. Conversely, engineering personnel will request a maximum figure for construction purposes and hold the informant to it. The question is raised again and again when circumstances make it necessary to put two hundred monkeys, for example, in a cage intended for one hundred. Erratic arrivals of monkey shipments or varying production schedules create inventory extremes. These extremes, because of fixed cage facilities, result in the practice of overcrowding. It is axiomatic to the science of husbandry that penalties are imposed when species ranging from bacteria to humans are overcrowded. Without resorting to figures, it may be stated that, in one large primate colony, statistical accounts of mortalities experienced during crowded versus uncrowded periods portray an unfavorable effect. Other deleterious influences, such as disease and social disorder, may be recognized individually.

Disease, as with social conflict, is promoted by and finds nourishment in the crowded milieu. To those who have witnessed the habits and nature of contact of colonized monkey groups the probability of disease transmission is unquestionable. The rapid progression of tuberculosis and certain enteric problems among congregated primates confirms the excellence of the condition of crowding for the promotion of disease. The gang cage provides an environment conducive to every mode of disease transmission. It also provides the carrier state, the susceptible individual, and the gamut of infectious agents. Our aim, it appears, should be one of segregating or at least the opposite of crowding—namely, dilution.

More subtle, but not without significance, are the societies of infrahuman primate groups under the forced conditions of colony husbandry. The extent and influence of social hierarchies among the forms of primates in question have been adequately reported.[1,2] Characteristically organized societies, strongly

hierarchical, are easily recognized among the Catarrhine monkeys. Dominance is invariant and always unidirectional. Maslow[2] describes Catarrhine dominance as rough, brutal, and aggressive. "It is a powerful, persistent, selfish urge, expressed in ferocious bullying, fighting and sexual aggression. The subordinate is completely afraid of the dominant animal to the point of being terror-stricken and cowed. The weak or sick are often killed by dominant personalities who seek to take advantage of opportunities. The very subordinate animal is in danger of starving if the supply of food is limited. The behavior of a subordinate shows the effect of the social relationship of brutal dominance and his behavior is oriented to his overlords. He will avoid the overlord; and look around before eating, playing, or exploring."

Such accounts as the above draw our attention and respect to the social conflicts potentially present in any gang-cage situation. Among certain groups of Cercopithecoids maintained in reasonably small groups, the brutality of fighting accounts for losses up to 30 per cent. Mortalities accrue as a result of the aforementioned aspects of colony or gang-cage methods. In the presence of crowding and social conflict, rest and nourishment are often restricted, but exposure to infection is certainly provided. Consequently, the strong become stronger and the weak become weaker.

The ultimate step in eliminating the influences of social competition and disease and to dilute rather than to crowd is to cage monkeys individually. This approach in a colony that inventories three thousand animals cannot be accepted, however, without serious economic consideration. The initial expense of a large number of cages and the cost of labor involved in the husbandry program must be acknowledged. An exceptional challenge is on hand, then, to develop a program of primate maintenance that includes such major considerations as: cost of caging, maintenance expense, disease control, safety, and practicability. In a basic analysis monkey husbandry on a large scale is not unlike flock management of poultry. Major aspects of flock management and primate colonies have analagous problems. In recent years the poultry industry has developed a system of individual caging for laying hens.[3-6] The success of this system led us to ask the following question. If an individualized system of keeping laying hens, otherwise kept as a flock, resulted in a successful practice, could a colony of monkeys be maintained satisfactorily under similar conditions? Such a study has been undertaken, utilizing an adapted layer cage, with the aim of eliminating the gang-cage system.

Groups of *Macaca mulatta* monkeys have been maintained for varying periods of time in these lightweight cages. One major group of three hundred animals has been maintained for two months. The results with respect to the original intent have been gratifying. Success with respect to the health and well-being of the caged monkey has been established. Secondary benefits of the system have become obvious, and the significant labor-saving features of the poultry system are likewise applicable to the maintenance of large groups of monkeys similarly caged (FIGURE 1).

One of the virtues of these lightweight cages is the flexibility of the component features. Such items as the method of feeding, watering device, clean-out system, and cage dimensions or material may be altered. A linear arrangement is essential, however, for maximum labor efficiency. Cage surfaces are not

cleaned during the confinement period. Continual contact by the monkey keeps the surfaces clean and shiny. After the groups in each ward are removed for use, the area is thoroughly hosed down and subjected to beta-propriolactone vapor (FIGURE 2).

FIGURE 1.

FIGURE 2.

It is necessary to point out that the control of environmental conditions in an area designed for such a cage system must be well planned. It is also obvious that there is restriction of activity in the use of this cage. We have watched closely for any ill effects from this restriction. Monkeys kept six to eight weeks

may show a slight muscular incoordination upon release into a larger area. The condition is transient and disappears after several minutes of running or other activity. Although the original use for these cages was for a confinement period of three or four weeks it is now felt that much longer periods of confinement are possible (FIGURE 3).

In summarizing the advantages of this system, the individual cage described (1) permits segregation to any degree desired; (2) reduces transmission of disease; (3) eliminates aggressive fighting or bullying; (4) eliminates restrictive social influences; (5) maintains companionship; (6) permits close inspection of

FIGURE 3.

each animal; (7) permits the use of drugs on a mass or an individual basis; (8) reduces handling, thus promoting safety; (9) reduces the cost of supply items; and (10) lowers the initial cage expense. Moreover, labor cost, through automatic watering, feeding, and cleaning, is estimated to be one-quarter that required for gang caging or other individual monkey cage systems.

The effects of crowding, disease and social conflict are recognized liabilities in the gang-cage system of infrahuman primate husbandry. In preliminary studies the use of a lightweight cage, adapted from the poultry layer-cage system, provides economy, segregation, simplicity, and a possible solution of gang-cage problems.

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THE ROLES OF INFECTIOUS AND NONINFECTIOUS DISEASES IN MONKEY HEALTH

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Introduction

During August 1958, a program designed to investigate the diseases of monkeys was undertaken jointly by the School of Veterinary Medicine, University of Pennsylvania and Merck Sharp & Dohme.

The purposes of this program were twofold: (1) to define the problems responsible for the deaths of captive monkeys in order that suitable corrective measures might be instituted; and (2) to add as much information as possible to the biological pool of knowledge.

During the early part of the investigation, it was found that mortality was a complex problem and that, as other workers in the field had suspected, it revolved around a "vicious" cycle of malnutrition, stress, and infectious disease.

In the discussion that follows, each aspect of the cycle will be treated separately before a correlation of the interrelationships is made.

Materials and Methods

Material for the investigation was obtained from a dynamic colony of approximately 2,000 *Macaca philippinensis*, *Macaca mulatta*, and *Cercopithecus aethiops sabaeus* monkeys housed at Merck Sharp & Dohme, West Point, Pa.

A large proportion of these animals were new arrivals to this country from their natural habitat. The duration of existence after arrival varied from 24 hours to approximately one year and averaged about one week.

Five hundred consecutive autopsies were performed on dead or euthanized moribund monkeys. The first one hundred and thirty-five post-mortems were complete for all systems except the nervous system. The main purpose of the first series was to define, in a general manner, the major and contributory conditions or diseases responsible for losses.

From this point on, post-mortem examinations were used as a tactical weapon, especially during an epidemic. The purpose then was to find the major cause or causes of death and to relate the pathology found with a specific etiological agent in order that proper corrective measures might be instituted rapidly. Many of these autopsies were extremely thorough but, because of extenuating circumstances, others were incomplete for some organ systems.

Organ and Body Weights

At the time of autopsy, all of the animals were weighed accurately to the nearest one-hundredth kilogram. Each organ was weighed to within one-tenth gram. The weight of any organ with gross pathological changes was not included in the data.

The organs were prepared for weighing as follows:

Heart. The pericardial sac was removed. The organ was opened in the manner described by Coffin,[1] and blood and extraneous material were removed by washing.

Liver. The diaphragm and loose fascia were removed. The gall bladder was opened and the organ washed thoroughly before weighing.

Kidneys. The capsule was removed and all vascular and fibrous attachments trimmed as closely as possible at the hilus.

Spleen. The splenic omentum and blood vessels were removed in their entirety.

Pancreas. The organ was dissected from the wall of the duodenum and trimmed of extraneous fat and fascia.

Adrenal glands. Immediately upon removal from the body, the glands were fixed in 10 per cent isotonic buffered formalin solution* to facilitate cleaning. After fixation, as much fascia as possible was trimmed away.

Histology. Material for histological examination was fixed in 10 per cent isotonic buffered formalin and prepared by Autotechnicon.† Sections were cut at a setting of 6μ and stained with hematoxylin and eosin.

Bacteriology. Whenever possible, specimens for examination were taken from euthanized moribund animals. Fecal swabs were placed in selenite broth (Difco) and after one hour were streaked on SS agar (Difco). All other material was streaked on plates containing blood agar base (Difco) with 5 per cent sheep blood.

Sedimentation rates and hematocrit. Oxylated blood (ammonium and potassium oxylate[2]) was placed in a Wintrobe hematocrit tube and the sedimentation rate was recorded after 1 hour. The tubes were then centrifuged (International PR-1) for 30 min. at 3,000 rpm and the hematocrit read.

Total Serum Protein

All samples were run in duplicate.

Total nitrogen: the U.S.P. procedure was followed except for digestion, which was for 2 hours.

Nonprotein nitrogen: the protein was precipitated with 10 per cent trichloroacetic acid and passed through Whatman No. 1 filter paper. The paper was then washed 3 times. The nitrogen content of the filtrate was determined by the U.S.P. Standard Kjeldahl Method for nitrogen determination.

Results

Infectious Disease

In general, the two major causes of death within the colony were bronchopneumonia and enteritis. The number of deaths attributable to each cause varied considerably from time to time and depended more on the species composition of the colony than on the season of the year.

Enteritis was more common in all species when large numbers of *M. mulatta*

* One per cent by volume of 0.1 molar solution of dibasic sodium phosphate.

† Technicon Company, Chauncey, N.Y.

were entering the colony. This was probably due to contamination of the colony as a result of the high incidence of *Salmonella* and *Shigella* carriers among *M. mulatta*[3] upon arrival. Bronchopneumonia was the major problem when the colony was composed primarily of *M. philippinensis* and *C. aethiops sabaeus* and often was an important factor in all three species upon arrival and shortly thereafter. It was, however, much more amenable than enteritis to prophylactic and therapeutic measures (Fegley and Sauer, this monograph). Therefore, while sporadic deaths occurred from bronchopneumonia, epizootics were infrequently encountered.

Table 1 summarizes the major causes of death based on the first 135 autopsies. At this time the colony was composed of approximately 70 per cent *M. philippinensis*, 29 per cent *M. mulatta* and 1 per cent *C. aethiops sabaeus.*

Enteritis

A study was made on 4,249 *M. mulatta* and 2,283 *M. philippinensis* received at the colony. Intestinal samples from 221 (3.5 per cent) of these animals were examined for the presence of bacterial pathogens. Some samples were obtained

Table 1

Incidence of the Four Major Disease Processes Occurring in the Colony (In Percentages)

Disease	Major cause of death	Contributory cause of death	Total incidence
Bronchopneumonia	33	16	49
Gastroenteritis	20	23	43
Malnutrition	23	52	75
Stress	1	62	63

at the time of arrival, some following antibiotic therapy, and others at the time of post-mortem examination. *Shigella flexneri* types 2, 3, and 4 were isolated from 21.2 per cent of the monkeys in the following distribution: *Sh. flexneri* type 3, 57.5 per cent; *Sh. flexneri* type 2, 36.0 per cent; and *Sh. flexneri* type 4, 6.5 per cent.

Of 36 cultures taken from rhesus monkeys with gross lesions of colitis and typhlitis, isolations of *Shigella* organisms were obtained from 29 (80 per cent).

Only two *M. philippinensis* were found to harbor *Shigella* organisms. This was 3.1 per cent of the 64 samples taken from this species. Twenty-four per cent of the samples were positive for *Salmonella* species. Although not all cultures were checked for group specificity, all but 1 tested were from *Salmonella* group E. One culture of *Salmonella typhimurium* was isolated from a shipment of *M. philippinensis.*

The pathology of *Shigella* and *Salmonella* infections in monkeys has been described by Gvazava,[4] Fairbrother and Hurst,[5] and Preston and Clark,[6] Cass,[7] and summarized by Ruch.[8]

The gross changes observed in the colon and cecum included thickening of the wall by edema, subserosal petechiae, mucosal hyperemia, and varying degrees of mucosal hemorrhage. Discrete ulcers were not observed.

The character of the lumen contents varied considerably and was frequently described as being of pea-soup consistency, watery, watery and bloody, catarrhal, catarrhal and bloody.

Microscopically, mucosal and submucosal hyperemia was usually intense. Lymph vessels of the mucosa and submucosa and lymph sinuses surrounding lymphoid follicles were distended and prominent. Edema was variable, as were the amount and character of cellular exudation that usually consisted of lymphocytes, plasma cells, histiocytes, and occasional polymorphonuclear leukocytes. Mucinous degeneration and dilatation of the crypts was most constant.

An interesting pathological lesion was observed in the colon and cecum of 9 monkeys. *Shigella flexneri* was isolated from the lumen contents of 4 of these. The other 5 were negative for enteric pathogens.

In these cases the cecum and colon were usually empty or at most contained a scant amount of thick, tenacious, grayish mucus. The wall was thickened and tense. The mucosa was dry and sticky, diffusely reddened, and covered with a thin, grayish, rather diffuse sheet resembling lens paper in color and general appearance. Light scraping removed this, leaving an intensely reddened surface.

Histological sections were characterized by thrombosis of submucosal capillaries and venules and diffuse fibrinous inflammation, with superficial necrosis of the mucosa (FIGURE 1*a*). Intranuclear type-A inclusion bodies were seen in epithelial cells of the crypts in three of these cases (FIGURE 1*b*). Cellular exudation was marked, especially in the mucosa, and consisted almost entirely of polymorphonuclear leukocytes.

Similar lesions, without reference to inclusion bodies, have been described by Anderson[9] in human cases of shigellosis.

Respiratory Disease

Bronchitis with associated atelectasis and emphysema was frequently observed upon microscopic examination of lung tissues and was the most common lesion affecting the respiratory system. It was not unusual to find this condition even in lungs that were apparently normal upon gross examination.

Bacterial pneumonia was the most serious disease encountered with the respiratory mechanism and, as stated earlier, was one of the 2 major causes of death within the colony. Upon bacteriological examination of 32 infected lungs, the following organisms were isolated: *Pneumococcus sp.* (13), *β-hemolytic Streptococcus* (11), *Staphylococcus* (9), *α-hemolytic Streptococcus* (1), and *Klebsiella pneumoniae* (1).

With few exceptions, the lesions both grossly and microscopically were patchy in distribution to form a lobular or bronchopneumonia. Coalescence of lesions with the production of large areas of consolidation was common.

Giant cell pneumonias were observed in one *M. mulatta* and one *C. aethiops sabaeus* monkey. This condition was described first in cynomolgus monkeys by Habermann and Williams.[10] The microscopic lesion was characterized by an interstitial pneumonitis with large multinucleated foreign-body-type giant cells (FIGURE 2). Eosinophilic inclusion bodies were seen within nuclei of epithelial cells of the bronchial mucosa (FIGURE 3), but not within nuclei of giant

cells. Cytoplasmic inclusion bodies, while present, were not prominent. This was possibly due to the degenerated condition of the tissue.

A case of pneumonia was observed in one female *M. philippinensis* monkey that was similar to the interstitial plasma cellular pneumonia of human in-

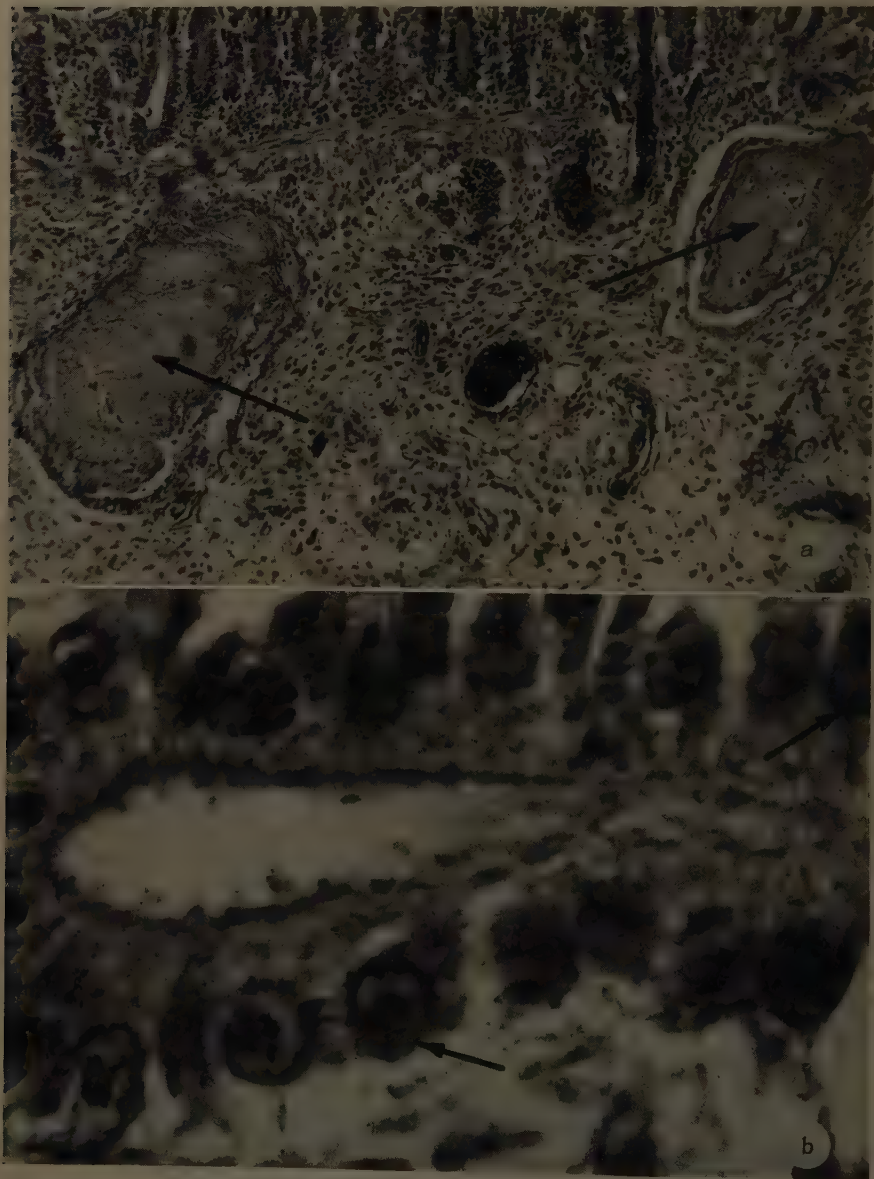

FIGURE 1. (*a*) Intestine showing thrombosis of submucosal veins (*arrows*). Hematoxylin-eosin. ×86. (*b*) Crypt of intestinal mucosa showing intranuclear inclusion bodies (*arrows*). Hematoxylin-eosin. ×2500.

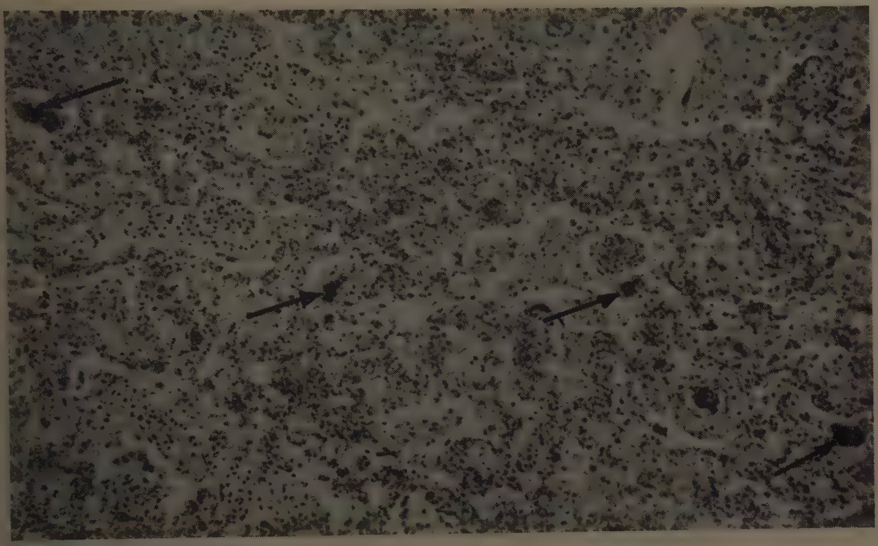

FIGURE 2. Interstitial pneumonitis with large multinucleated foreign-body-type giant cells (*arrows*). Hematoxylin-eosin. ×125.

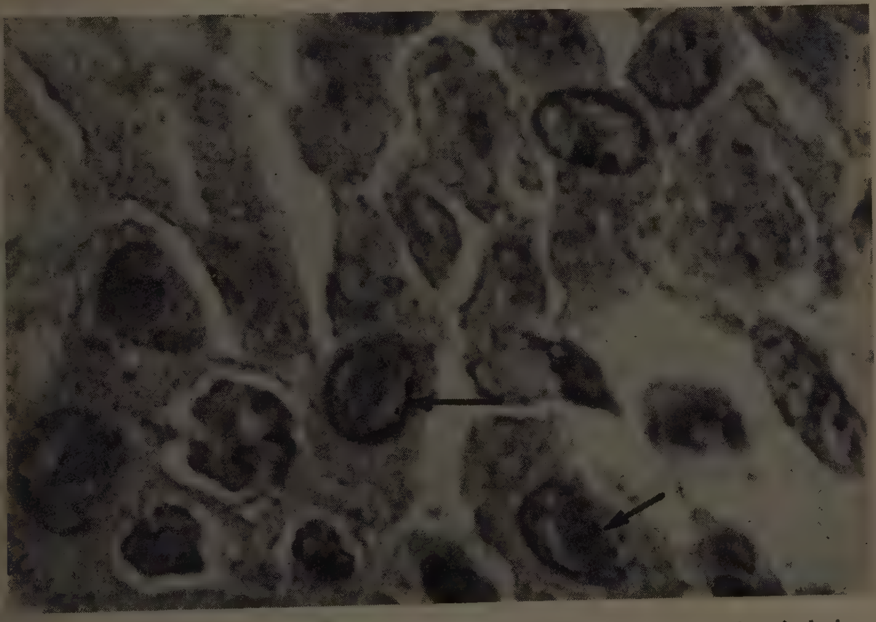

FIGURE 3. Bronchial mucosa in giant cell pneumonia showing intranuclear inclusions. Hematoxylin-eosin. ×2500.

fants[11,12] (FIGURE 4). However, the parasite responsible for the disease in humans (*Pneumocystis carinii*) could not be found in the monkey tissues.

Massive pulmonary infarction was suspected in another *M. philippinensis* female. The lesion was not recognized as such at autopsy and it was only in retrospect that such a conclusion was made. Post-mortem examination revealed a suppurating compound fracture of the distal portion of the left tibia and multiple abscesses of the liver. The distal half of the left intermediate and inferior lobes of the lung was swollen and cream-colored, while proximal portions were mottled with red and gray. On histological examination of the lung, extensive irregular areas of coagulation necrosis were sharply demarcated

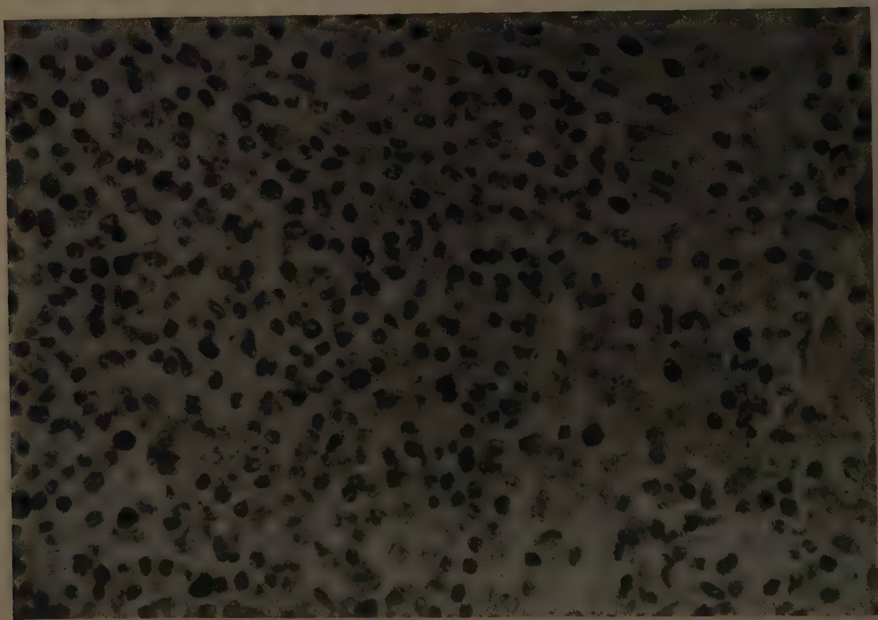

FIGURE 4. Interstitial plasma cellular pneumonia. Hematoxylin-eosin. ×500.

from the remaining viable but pneumonic parenchyma (FIGURE 5). Multiple thrombi were seen in arteries and arterioles of the viable portions adjacent to the necrotic areas.

Noninfectious Disease

During the course of the investigation, it became desirable to have certain information such as organ weights, serum protein, and hematocrits. In order to evaluate this information, it then became necessary to have standards for purposes of comparison. To the best of our knowledge, standards were not available for normal monkeys of the species with which we were most concerned.

Upon arrival in this country the average monkey cannot be termed "normal" by usual standards. Most individuals suffer from some degree of malnutrition and often one or more infectious diseases. In many forms of research and pro-

duction, the individual monkey is expendable, and most animals after arrival do not remain in the colony a sufficient length of time to become conditioned. When afforded reasonable care, however, the health of most of these monkeys, while not "normal," is compatible with life and the uses for which they are required.

Workers in certain fields are thus forced to use an unconditioned prototype monkey that, by usual standards, is not normal but, nevertheless, one that has within itself an average set of biological values.

It was decided, therefore, to determine some of the unconditioned prototype's values and use these as standards. By doing this, one encounters certain limitations. Because the average is obtained from the pooling of the poorest as well as the better individuals, the range about the mean becomes almost limitless, and most individuals, regardless of condition, will fall within the limitations of the range. It becomes evident that such standards can be used only for the comparison of group averages. Nevertheless, we now have a standard by which we can compare a group of monkeys and quantitatively record those areas in which they are deficient or superior. For certain data, ranges can be speculated on the basis of similar data from other species. In such cases individual animals can then be qualitatively compared and judged.

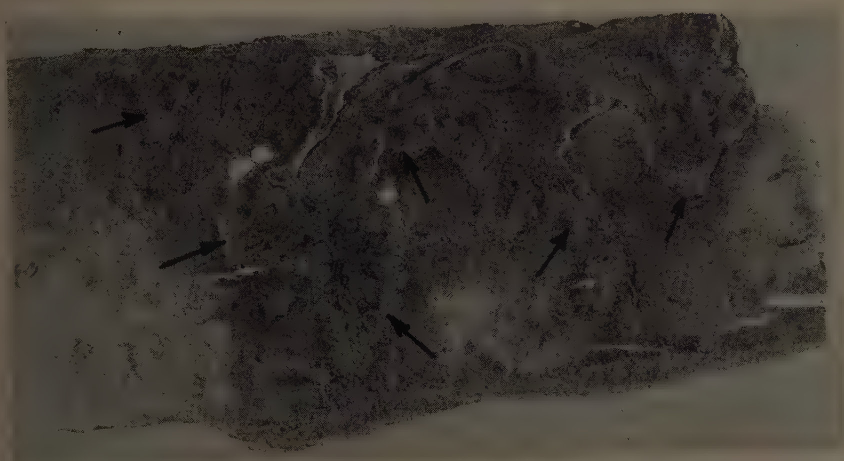

FIGURE 5. Massive pulmonary infarction. Extensive irregular area of coagulation necrosis (*below*) is sharply demarcated (*arrows*) from pneumonic parenchyma (*above*). Hematoxylin-eosin. Gross specimen.

Malnutrition

Malnutrition is one of the major factors involved in the mortality of imported monkeys, for it is a primary or contributing cause in 75 per cent of all deaths. Its importance cannot be overemphasized, for sound nutrition is basic if an animal is to respond adequately to such ever-present innate and environmental factors as growth, reproduction, stress, and infection.

Much effort and research have been expounded on malnutrition, and it is well

to summarize some of the general information concerned with this problem. Anyone wishing to pursue the subject should consult the works of Jackson[13] and Follis.[14]

Deficiencies under natural conditions rarely, if ever, occur singularly. If a diet lacks one factor, it is probably deficient or imbalanced in several others.

The extent to which an animal can resist the effects of malnutrition varies with the individual, the age, the state of nutrition, the type of inanition, and the environmental conditions. The capacity to endure is greatest in older animals and is enhanced if water is available and if the animal body contains stored food, especially fat. Exercise and low temperatures reduce endurance.

The lower resistance of young animals is due to higher requirements resulting from higher metabolism, growth, maintenance, and an apparent deficit in stored reserves. Thus, according to Jackson, it is possible for a young animal to starve to death while on a diet adequate to maintain body weight.

The monkeys with which we are concerned spend approximately 4 to 6 weeks in transit from their natural habitat to the laboratory. During this period they receive a diet which, under the best environmental conditions, would be dubiously adequate. They are caught, handled, restrained in crowded cages, subjected to repeated shuffling of hierarchies, exposed to extreme fluctuations of temperature and pressure, and placed in peril of a host of infectious agents. All of these factors interfere with nutrition in one or more ways by restricting intake, absorption, and assimilation or by increasing metabolism and thus nutritional requirements.

With two exceptions, the criteria by which inanition was judged were not constant findings in each individual. On the contrary, some athreptic individuals were even above the prototype average in certain respects and extremely low in regard to others.

Observed Effects of Malnutrition

Adipose tissue. A constant and one of the most striking changes of inanition was loss of fat from the depots. Gross depletion was evidenced first in the subcutaneous areas of the trunk, but was soon followed by loss in the omentum and perirenal and retroperitoneal areas.

Whereas the subcutaneous adipose tissue of a well-conditioned monkey approached one-half cm. in thickness, the same tissue was virtually absent in the marasmic animal, and the skin appeared to lie directly over the muscle and fascia. Similarly the omentum of conditioned monkeys was ladened with fat and was greasy to the touch, while the omentum of the malnourished monkey was transparent and veil-like (FIGURE 6).

The capacity to store fat varied somewhat with the species. The *M. mulatta* had a tendency to carry more fat and was easier to condition than the *M. philippinensis.*

Thymus. Gross atrophy of the thymus was constant and often appeared to be complete. It was not unusual to be unable to distinguish thymic tissue at autopsy.

Microscopically, there was a paucity of lymphocytes and a relative increase in stroma (FIGURE 7). The cortex was thin and rarefied and, in some instances, was indistinguishable from the medulla.

Loss in organ weight. Weight loss of individual organs may be expressed as actual weight loss or as relative weight loss. The actual weight loss is obtained by comparing the weight of an atrophic organ with the average normal weight of the same organ. The relative weight loss is obtained by comparing the percentage of total body weight of an organ with an average normal percentage.

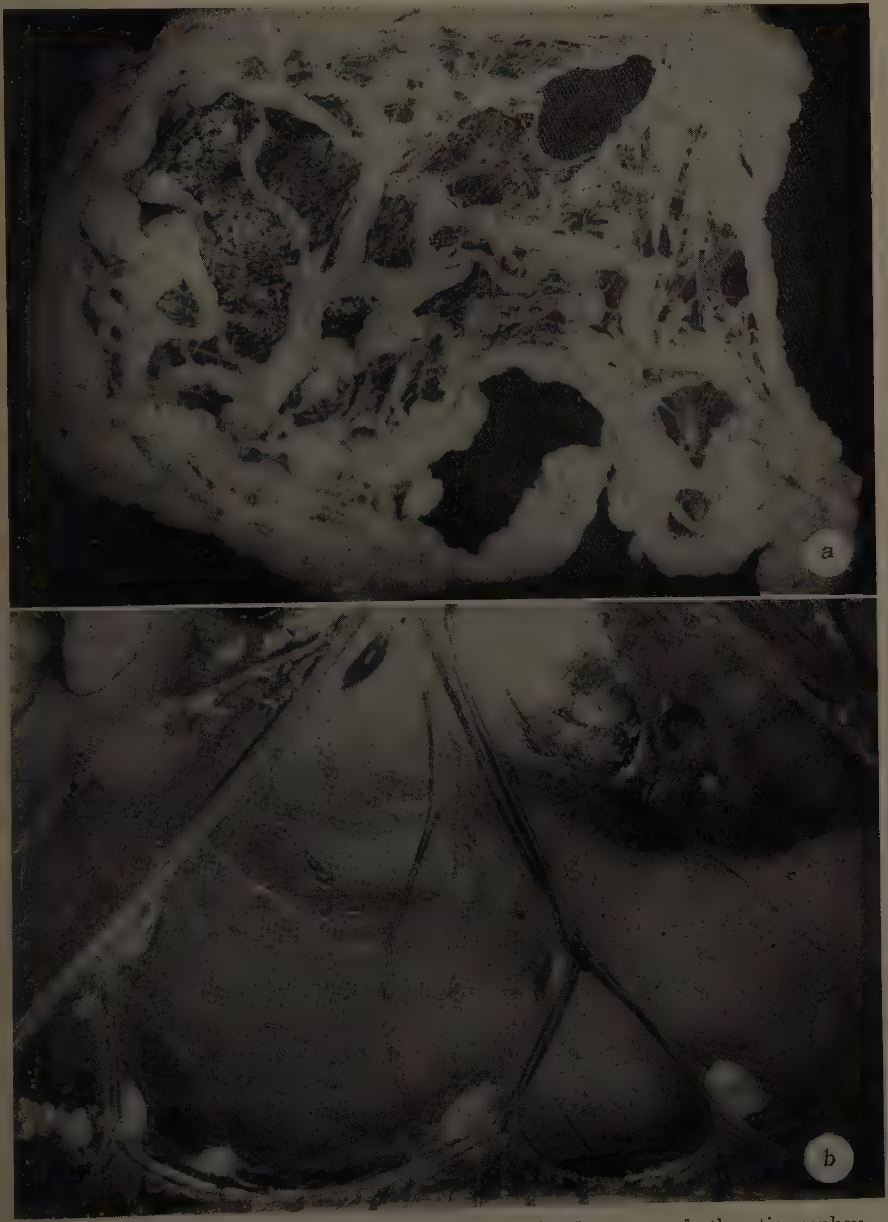

FIGURE 6. (*a*) Omentum of conditioned monkey. (*b*) Omentum of athreptic monkey.

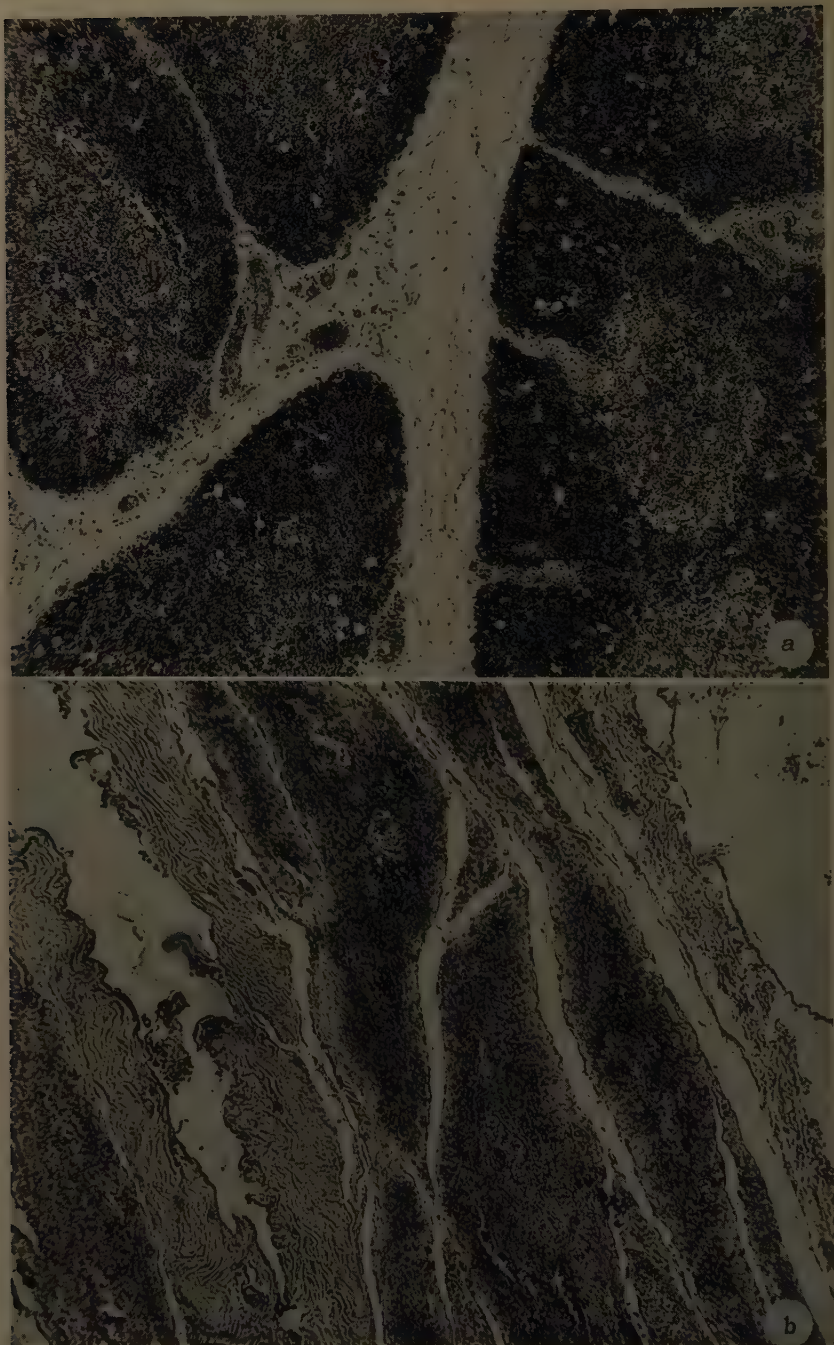

FIGURE 7. (*a*) Thymus of conditioned monkey. Hematoxylin-eosin. ×50. (*b*) Thymus of athreptic monkey. Hematoxylin-eosin. ×50.

Loss of total body weight in a malnourished animal comes about first by depletion of stored reserves, especially fat and, second, by atrophy of various organs and tissues. It is conceivable, then, in the early stage of inanition with loss of reserves and through loss of total body weight without loss of organ parenchyma that the weight of an organ could increase relative to total body weight.

Data on organ weights are available in the literature for some species of primates.[15-18]

TABLES 2, 3, 4, and 5 present similar information concerning the unconditioned prototypes of *M. philippinensis* and *C. aethiops sabaeus* species.

TABLE 6 compares the average relative weights of several organs from 17 malnourished *M. philippinensis* with the average relative weights of the unconditioned prototype. This group did not contain all of the malnourished animals encountered, but represents only those monkeys in which inanition was considered a primary and not merely a contributory cause of death.

TABLE 2
AVERAGE WEIGHT OF VARIOUS ORGANS IN SEVERAL WEIGHT CLASSES OF *MACACA PHILIPPINENSIS* (UNCONDITIONED PROTOTYPE)

Organs	0.5 to 1.00 kg.		1.01 to 1.50 kg.				1.51 to 2.00 kg.				2.01 to 2.50 kg.	
	Male & female mixed		Male		Female		Male		Female		Female	
	Gm.	No. observations	Gm.	No. observations	Gm.	No. observations	Gm.	No. observations	Gm.	No. observations	Gm.	No. observations
Heart	3.7	6	4.62	6	4.9	11	6.5	11	6.4	12	9.1	8
Liver	26.9	6	38.4	7	42.4	11	58.7	11	62.3	12	78.2	8
R. kidney	3.4	6	4.3	6	4.7	10	6.4	11	6.1	12	8.9	7
L. kidney	3.4	6	3.7	6	4.5	10	6.2	11	6.4	12	8.8	7
Spleen	1.9	4	2.6	5	2.6	9	3.3	7	3.6	11	4.5	7
Pancreas	1.5	1	2.1	1	2.7	2	3.1	1	3.1	3	5.6	3

Spleen. Conclusive gross atrophy of the spleen was not observed. The average relative weight of the spleens of a group of malnourished *M. philippinensis* (TABLE 6) was 11.5 per cent below the unconditioned prototype average. In this group, on the other hand, 4 of 16 individuals had spleens that were heavier than the prototype average.

Histologically, atrophy was a common but by no means constant finding. In such cases there was an actual loss of lymphocytes and a relative increase in the stroma. The malpighian corpuscles were decreased in size and less distinct. The number of erythrocytes present in the red pulp was variable.

Liver. The liver, like the spleen, showed much individual variation in response to inanition. Macroscopically, 10 livers from the malnourished group were not remarkable; 6 were dark red in color and congested but had extremely sharp edges, and one had a distinctly brown cast. The average weight loss for the group was 11.4 per cent (TABLE 6), but 4 of the 17 showed an increase in

weight as compared to the unconditioned prototype average. The greatest single decrease in weight was 44 per cent.

Histological sections were available for 15 of the 17 livers, and 8 showed evidence of atrophy of the parenchyma (FIGURE 8), which chiefly affected the quantity of cytoplasm.

TABLE 3

AVERAGE PERCENTAGE BODY WEIGHT OF VARIOUS ORGANS IN SEVERAL WEIGHT CLASSES OF *MACACA PHILIPPINENSIS* (UNCONDITIONED PROTOTYPE)

Organs	0.50 to 1.00 kg.		1.01 to 1.50 kg.				1.51 to 2.00 kg.				2.01 to 2.50 kg.	
	Male & female mixed		Male		Female		Male		Female		Female	
	% and range	No. observations	% and range	No. observations	% and range	No. observations	% and range	No. observations	% and range	No. observations	% and range	No. observations
Heart	0.396 (0.358–0.516)	5	0.377 (0.330–0.404)	6	0.380 (0.291–0.587)	11	0.378 (0.291–0.565)	11	0.358 (0.259–0.467)	12	0.398 (0.322–0.445)	8
Liver	2.98 (2.61–3.62)	5	3.26 (2.60–3.95)	7	3.33 (2.58–4.49)	11	3.41 (1.99–4.98)	11	3.51 (2.86–4.14)	12	3.25 (2.34–4.26)	8
R. kidney	0.394 (0.305–0.414)	5	0.362 (0.271–0.500)	6	0.368 (0.293–0.462)	10	0.375 (0.294–0.534)	11	0.349 (0.287–0.390)	12	0.406 (0.318–0.494)	7
L. kidney	0.330 (0.284–0.442)	5	0.315 (0.248–0.441)	6	0.353 (0.286–0.437)	10	0.361 (0.253–0.534)	11	0.367 (0.276–0.500)	12	0.392 (0.318–0.472)	7
Spleen	0.210 (0.166–0.263)	4	0.233 (0.148–0.300)	5	0.205 (0.110–0.305)	9	0.195 (0.162–0.241)	8	0.210 (0.140–0.331)	11	0.203 (0.128–0.298)	7
Pancreas	0.221	1	0.210	1	0.203	2	0.206	1	0.188	3	0.249	3

TABLE 4

AVERAGE RELATIVE WEIGHTS OF ORGANS IN FIFTY-FOUR *MACACA PHILIPPINENSIS* OF BOTH SEXES AND ALL WEIGHT CLASSES (UNCONDITIONED PROTOTYPE)

Organ	Percentage of body weight	Range
Heart	0.378	0.291 to 0.587
Liver	3.33	1.99 to 4.98
R. kidney	0.372	0.271 to 0.534
L. kidney	0.357	0.248 to 0.534
Pancreas*	0.214	0.153 to 0.255
Spleen	0.218	0.110 to 0.331
Right adrenal†	0.0196	0.0117 to 0.0357
Left adrenal†	0.0297	0.0187 to 0.0420

* Average percentage of 8 glands.
† Average percentage of 15 glands.

Pericardial and peritoneal effusion. These signs were by no means constant, but occurred with such regularity, either singularly or together, as to become obvious. For example, in the group of 17 athreptic *M. philippinensis*, 3 had

pericardial effusion, 4 had peritoneal effusion, and 3 had both. Of the 7 remaining animals, 4 hearts could not be observed because of hemorrhage resulting from antemortem cardiac puncture for blood.

The pericardial effusion, which varied in amount from 2 to 6 cc., was clear or slightly amber-colored and serous.

In the peritoneal cavity, effusion was not observed as a massive collection of fluid. The viscera were frequently discribed as "soggy" or "wet," as if dipped in water, and small "puddles" of a clear or slightly amber fluid were noted on the floor of the abdominal and pelvic cavities upon removal of the viscera. The maximum collection amounted to approximately 30 cc.

Table 5

Average Weights and Average Relative Body Weights of Various Organs in Eleven Female *Cercopithecus aethiops sabaeus*

(Unconditioned Prototype)

Organ	Av. weight in grams	Percentage of body weight	Range
Heart	7.5	0.461	0.356 to 0.711
Liver	85.3	4.91	4.17 to 6.06
R. kidney	7.3	0.420	0.331 to 0.514
L. kidney	7.2	0.437	0.331 to 0.524
Spleen	3.0	0.211	0.125 to 0.250

Table 6

Relative Weight Loss of Organs of Seventeen Malnourished *Macaca philippinensis*

Organ	Av. percentage body wt., unconditioned prototype	Av. percentage body wt., malnourished	Relative wt. loss (%)
Heart	0.378	0.350	7.4
Liver	3.33	2.95	11.4
Spleen	0.218	0.193	11.5
R. kidney	0.372	0.338	9.1
L. kidney	0.357	0.320	10.0

Anemia. Hematocrit values for conditioned *M. philippinensis* monkeys and the *M. mulatta* and *M. philippinensis* unconditioned prototypes are shown in Table 7. Krise *et al.*[19] have reported the hematocrit for the normal male *M. mulatta* monkey as 45.47 per cent, with a range of 35 to 53 per cent.

It appears from these data that the prototypes of both species show a relative but not a significant anemia. A species variation is also apparent in that average values for *M. philippinensis* monkeys are below those of comparable *M. mulatta* monkeys.

Table 8 shows the hematocrit values for a group of Philippine cynomolgus monkeys and a group of Malayan cynomolgus monkeys. Both groups were clinically malnourished and, from the figures, it is obvious that they were anemic.

Total serum protein. The total serum protein values for the unconditioned

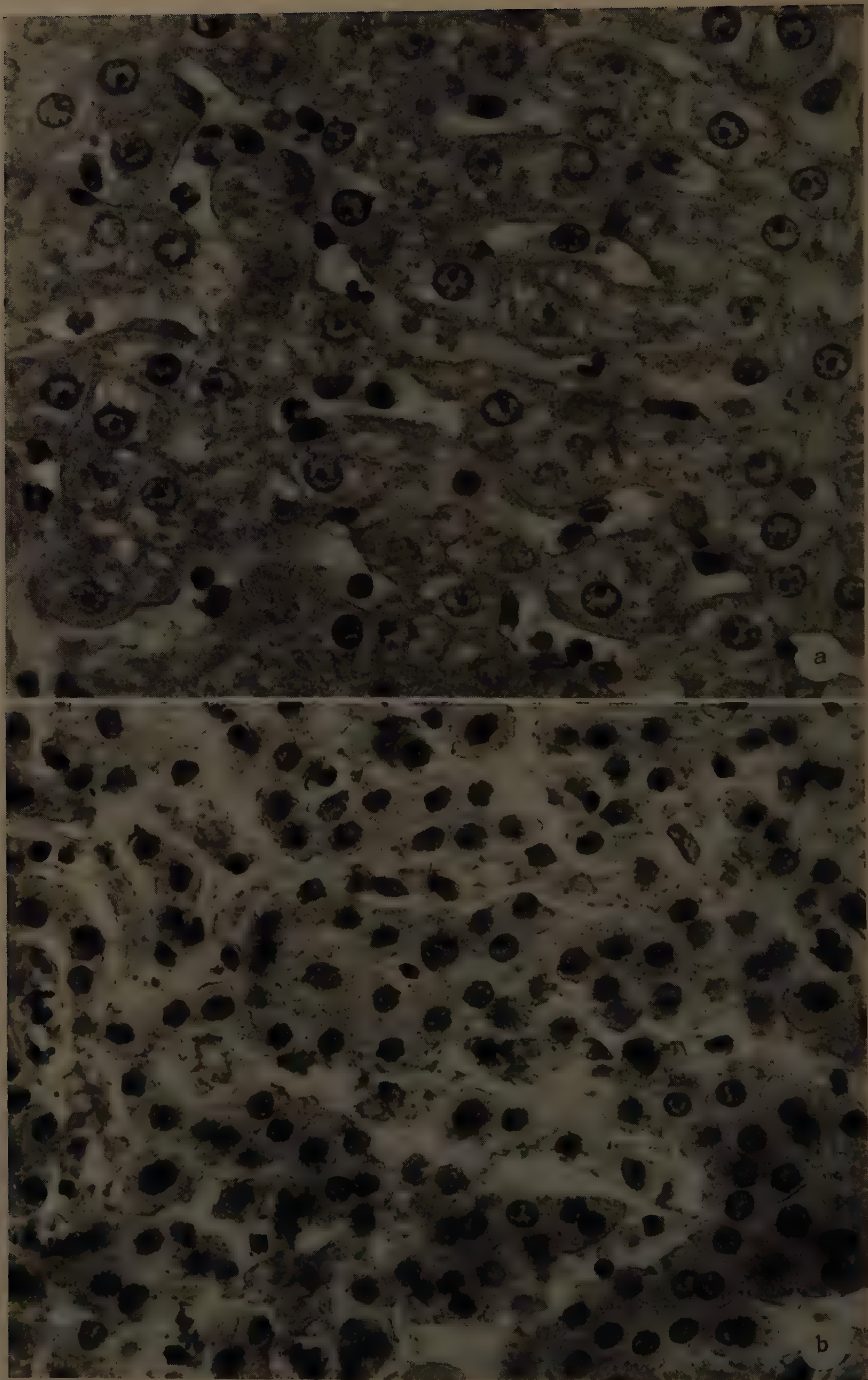

FIGURE 8. (*a*) Liver of conditioned monkey. Hematoxylin-eosin. ×544. (*b*) Liver of athreptic monkey. Hematoxylin-eosin. ×544.

prototypes of *M. philippinensis*, *M. mulatta*, and *C. aethiops sabaeus* monkeys are shown in TABLE 9. Because these values were obtained from the prototype, a normal range could not be established. The average values, however, are very close to those found in the human, and the normal human range is 6.0 to

TABLE 7
HEMATOCRIT VALUES FOR *M. PHILIPPINENSIS* AND *M. MULATTA*

	Hematocrit (%)	Range (%)	No. observations
M. philippinensis (prototype)			
Male & female combined	34.1	19 to 57	66
Male	36.1	28 to 57	23
Female	33.0	19 to 52	41
M. philippinensis (conditioned)			
Male & female combined	37.9	35 to 42	14
Male	38.8	37 to 41	5
Female	37.4	35 to 42	9
M. mulatta (prototype)			
Male & female combined	40.7	27.5 to 55.0	72
Male	41.4	27.5 to 55.0	32
Female	39.7	31.0 to 50.0	38

TABLE 8
HEMATOCRIT VALUES FOR TWO CLINICALLY MALNOURISHED GROUPS OF MONKEYS

M. philippinensis		*M. irus*	
Monkey no.	Hematocrit (%)	Monkey no.	Hematocrit (%)
89	22	131	27
90	22	132	27
91	28	133	25
92	32	134	25
93	29	135	25
94	32.5	136	29.5
95	27	137	26.5
96	32	138	20
97	24	139	31
98	33	140	25
		141	29.5
Average	28.1	142	24.5
		145	27.5
		146	25
		Average	26.3

7.8 gm. per 100 cc. of serum.[20] If the monkey is found to have a similar range, then 15 per cent of the present series will be subnormal.

This, however, is speculation. Further studies are being conducted on an additional 160 monkeys, of which a small percentage is conditioned.

Stress

Stress is probably one of the most intangible and difficult subjects to evaluate objectively. In spite of this, one cannot discount the importance of this factor

in monkey health. As Hans Selye once said, "... it must be admitted that 'stress' is an abstraction; but 'life' is also an abstraction, and yet, it could hardly be rejected as a worthless concept in biology."[21]

For the purposes of this study, gross and microscopic changes in adrenal morphology were the primary measure of the acute stress factor. The criteria used included cortical hypertrophy and hyperplasia, hyperemia (FIGURE 9), hemorrhage (FIGURE 10), and necrosis.

For purposes of comparison and interpretation, it may be said that those adrenal changes in the monkey are both quantitatively and qualitatively more striking than adrenal lesions seen in domestic animals under comparable circum stances.

TABLE 9
TOTAL SERUM PROTEIN VALUES FOR UNCONDITIONED PROTOTYPE
(Kjeldahl Method)

	Protein gm./100 cc. serum	No. observations
M. philippinensis		
Male & female combined	6.86	89
Male	6.80	29
Female	6.89	48
M. mulatta		
Male & female combined	6.87	92
Male	6.98	44
Female	6.65	40
C. aethiops sabaeus		
Male & female combined	6.97	8
All species & both sexes	6.85 S.D. ±0.89	191

SEDIMENTATION RATES

In the laboratory it is usually important to have at one's disposal monkeys that are healthy and free of infectious disease. A simple test that would indicate qualitatively the condition of a monkey would be of immeasurable benefit.

TABLES 10, 11, 12, and 13 indicate the results of the sedimentation rates obtained from 4 groups of monkeys. Two of these groups were composed of sickly, newly arrived specimens of *M. philippinensis* and *M. mulatta* monkeys. The other 2 groups, composed of the same species, had been conditioned on the premises.

From these results, it is our interpretation that a monkey with a sedimentation rate of less than 2 mm./hour could be considered, for most intents and purposes, as healthy. In view of the fact that 12 monkeys had high sedimentation rates and no gross evidence of disease, we hesitate to say that a high sedimentation rate necessarily indicates poor health.

This, in all probability, is not a failing of the test, but rather a failing of the

investigators to perceive the underlying pathology upon post-mortem examination.

FIGURE 9. Adrenal cortex. (*a*) Hyperplasia and hyperemia; (*b*) control. Hematoxylin-eosin. ×85.

Discussion

The problem of monkey health appears to be neither a simple nor a constant one, but rather a "vicious" cycle involving: (1) varying degrees of acute malnutrition; (2) a variety of infectious conditions; and (3) acute stress (FIGURE 11).

Upon which aspect of the cycle most emphasis should be placed is a matter of conjecture. While infectious disease is the dramatic and obvious cause of most fatalities, stress and malnutrition are insidious, predisposing factors.

To a certain extent, malnutrition is probably innate. After capture, the condition is aggravated by inadequate artificial diets. This is not difficult to

understand when we realize that at the source of supply adequate foodstuffs are often unavailable to feed humans, not to mention monkeys.

Age is another factor that contributes to the state of inanition. A large proportion of the monkeys shipped to this country are immature, and the increased requirements of growth add to the need for nourishment.

FIGURE 10. Adrenal gland. Massive hemorrhage in medulla. Hematoxylin-eosin. ×50.

Malnutrition reduces resistance to infectious disease and to nonspecific stressors, for the animal cannot adequately respond to the demands imposed by these factors.

Infectious disease, on the other hand, potentiates malnutrition by: (1) reducing appetite; (2) reducing the ability to compete; (3) reducing absorption; (4) interfering with assimilation; and (5) increasing nutritional requirements. As infectious diseases are in reality a type of stressor, they may lower resistance, under certain circumstances, to other forms of stressors such as cold or crowding.

This phenomenon, in terms of the general adaptation syndrome, is called "crossed sensitization."[22]

Nonspecific stress imposed by handling, shipping, crowding, and other such factors also potentiates inanition through such media as fear, competition, loss

TABLE 10

CORRECTED SEDIMENTATION RATES* OF 24 DISEASED *M. PHILIPPINENSIS*

Monkey no.	Sed. rate mm./hr.	Post-mortem findings
1	48	Negative
2	19	Bronchopneumonia
3	7	Negative
4	10	Bronchopneumonia
5	14	Bronchopneumonia, enteritis
6	Clotted	Bronchopneumonia, enteritis
7	34	Bronchopneumonia
8	16	Bronchopneumonia
9	23	Bronchopneumonia
10	32	Negative
11	2	Bronchopneumonia
12	5	Negative
13	8	Bronchopneumonia
14	28	Negative
15	18	Negative
16	2	Bronchopneumonia
17	13	Bronchopneumonia
18	4	Bronchopneumonia
20	14	Negative
21	10	Bronchopneumonia
65	8	Enteritis
66	43	Negative
67	19	Enteritis
68	8	Negative

* Corrected for mean prototype hematocrit.

TABLE 11

CORRECTED SEDIMENTATION RATES* OF 14 CONDITIONED *M. PHILIPPINENSIS*

Monkey no.	Sed. rate mm./hr.	Post-mortem findings
1	2	Negative
2	0	Negative
3	3	Negative
4	0	Negative
5	1	Negative
6	0	Negative
7	5	Negative
8	0	Negative
9	0	Negative
10	0	Negative
11	24	Tuberculosis
12	0	Negative
13	0	Negative
14	0	Negative

* Corrected for mean conditioned hematocrit.

Table 12

Corrected Sedimentation Rates* of 25 Diseased *M. mulatta*

Monkey no.	Sed. rate mm./hr.	Post-mortem findings
50	36	Hemorrhagic colitis
51	22	Colitis and typhlitis
52	12	Colitis and bronchopneumonia
53	40	Pseudomembranous colitis
54	24	Pseudomembranous colitis
55	22	Pseudomembranous colitis; bronchopneumonia
56	14	Colitis and bronchopneumonia
57	14	Colitis and typhlitis
58	51	Colitis and typhlitis
59	18	Colitis, typhlitis, and bronchopneumonia
60	46	Colitis
81	31	Bronchopneumonia
82	22	Enteritis
83	0	Negative
84	47	Enteritis
85	7	Negative
86	1	Negative
87	22	Negative
88	8	Negative
42	24	Colitis
43	40	Bronchopneumonia
44	6	Enteritis
45	0	Negative
46	16	Bronchopneumonia, enteritis
47	7	Bronchopneumonia

* Corrected for mean prototype hematocrit.

Table 13

Corrected Sedimentation Rates* of 26 Conditioned *M. mulatta*

Monkey no.	Sed. rate mm./hr.	Post-mortem findings
103	0	No autopsy
104	0	No autopsy
105	0	No autopsy
106	0	No autopsy
107	0	No autopsy
108	0	No autopsy
109	0	No autopsy
110	0	No autopsy
111	0	No autopsy
112	0	No autopsy
113	0	No autopsy
114	0	No autopsy
115	0	No autopsy
116	0	Negative
117	0	Negative
118	0	Negative
119	0	Negative
120	0	Negative
122	0	Negative
123	0	Negative
124	0	Negative
125	0	Negative
126	0	Negative
127	0	Negative
128	0	Negative

* Corrected for mean prototype hematocrit.

of appetite, nervous diarrhea and increased requirements. Also by "crossed sensitization," it lowers resistance to facultative pathogens such as streptococci, staphylococci, and *Shigella* organisms, as well as to other infectious agents.

In our opinion, malnutrition is a basic factor in the initiation of the "cycle." When man imposes additional stressors, facultative pathogenic organisms become opportunists, and catastrophe is at hand.

Summary

A comprehensive study of the diseases of monkeys was undertaken for the purposes of: (1) defining the causes of fatalities in captive monkeys; and (2) adding as much information as possible to the biological pool of knowledge.

M. philippinensis, *M. mulatta*, and *C. aethiops sabaeus* monkeys were used in the study. These animals were "unconditioned prototype" monkeys which,

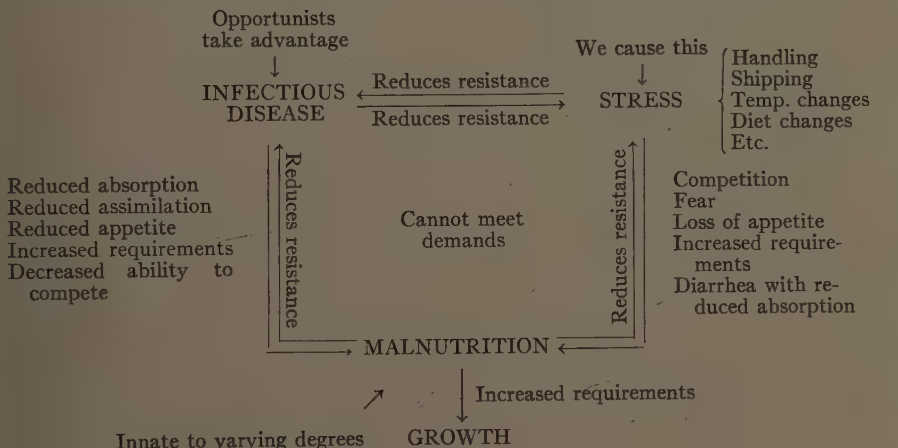

FIGURE 11.

by usual standards, are not normal, but which represent the average type of monkey received in the United States.

Values were obtained for organ weights, hematocrit, total serum protein, and sedimentation rates of the unconditioned prototype monkey and, in some instances, for the conditioned monkey.

Infectious disease, malnutrition and stress are described separately and correlated to form a "vicious" cycle that accounts for the majority of losses observed in monkey colonies.

Acknowledgments

We are indebted for serum protein analysis to John Grava and Joan Davitt;* for bacteriology to S. M. Zulick and L. F. Schuchardt;* for hematology to Carl Gabriel;* and for photography to Adolph Marfaing.†

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† The Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

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ORAL DISEASE IN PRIMATES

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The increasing use of primates for investigations of oral tissues is evident from a perusal of the literature. This is to be expected when one examines the many anatomic and physiological similarities found among the dentitions, supporting structures, and other oral tissues in nonhuman primates and humans. That many of these species are susceptible to the same oral diseases as is man enhances their value in the laboratory. An effort will be made in this presentation to cite the various pathological conditions that occur in the oral cavities of nonhuman primates.

Dentition

It is interesting to note that in 1735 Linnaeus, in classifying the animal kingdom, grouped man with ape and monkey on the basis of teeth, hands, fingers, and toes (Ruch, 1941). Linnaeus placed importance upon the teeth as a feature distinguishing primates from other forms; he originally defined a primate as "a quadruped with four parallel incisors, single canines, two pectoral mammae, hands, two complete clavicles and an arboreal habitat."

Since that time there have been several studies of dentitions with special emphasis upon both gross and microscopic malformations of the teeth (Williams, 1897). Colyer (1931 and 1936) points out the occurrence of enamel hypoplasia in conjunction with other malformed calcified tissues. He notes that the hypoplastic defect may be rows of pits or grooves affecting certain areas of specific teeth, especially the canines.

Schuman and Sognnaes (1956) studied microscopic developmental defects in teeth of 125 specimens in the primate group. They used shadow-cast collodion replicas and ground sections of the teeth for their documentation. They found that gross hypoplastic lesions were unusual; however, microscopic defects such as interglobular dentine and areolar dentine were frequently observed in the chimpanzee, orangutan, and gibbon teeth. Interestingly enough, the *Macaca mulatta* showed a relatively low incidence of developmental errors of any kind.

Dental Caries

During the past thirty years there have been a number of reports on the incidence of dental caries (James and Barnicot, 1949; Kazimiroff, 1939) in various nonhuman primates (Anderson and Arnim, 1934). The early studies attempted to document caries incidence in animals living in their natural habitat and in those in captivity. The studies of Colyer (1931 and 1936), Schultz (1935, 1941, 1944), and others (Granados, 1944; Mellanby, 1930 and 1934) point out the relatively low percentage of carious lesions among wild primates and the increase in incidence in the same genus in the captive state. It was noted also that the lesions in captive animals are found most often in

the premolar and molar teeth, the canines and incisors being less involved. There is also a suggestion that the maxillary teeth are more often attacked by caries than are the mandibular. Shaw *et al.* (1945*a*) and Shaw and Sognnaes (1955) point out that the criteria used by early investigators for determining a carious area may be inadequate: magnification and exploration of the lesions were much less accurate than was histological sectioning, whether by grinding or by use of a haıd-tissue cutting apparatus.

In an attempt to produce experimental dental caries, various combinations of diets have been utilized. Colyer (1936) felt that the susceptibility of man and of captive simians to caries that start in the enamel is due to the diet, especially the quantities of carbohydrate. Howe (1924*a* and *b*) attempted to produce caries by keeping monkeys on several types of diet, one deficient in ascorbic acid, a second lacking yeast, and a third without calcium. As Ruch (1959) points out, while these early studies are not clearly described and do not warrant a discussion of specific dietary deficiencies as a cause of caries, the statistics (twelve carious animals in forty-four) indicate a severity and frequency of dental disorder not found in surveys of caries incidence in colonies, although the incidence was not as high as that reported in a later study by Shaw *et al.* (1945*a* and *b*).

The interesting work of Rhinehart and Greenberg (1956), in which they studied the increased incidence of dental caries in *M. mulatta* on pyridoxine-deficient diets, gave additional evidence of nutritional factors. They noted a 45 per cent increase of caries in the deprived monkeys over those in the controls, and the lesions were larger and more rapidly progressing in the experimental group. We have examined the skulls of pyridroxine-deficient macaques and noted the high incidence of malocclusion. There were distinct alterations in tooth-to-tooth position, extreme crowding being prevalent. The higher incidence of caries in these monkeys may be due partly to the impaction of foodstuff that accompanies the malalignment of teeth (Breitner, 1943).

Shaw and his co-workers (1945*a*) have conducted many studies of dental caries in monkeys and have concluded: "The susceptibility of the rhesus monkey to tooth decay observed in this survey is sufficiently high to warrant the extensive use of this species in dental caries research. The high susceptibility reported is apparently due to the type of dietary regimen. It would appear from the data at hand that diets which contained high amounts of sucrose produced a greater frequency of dental caries in the monkey than natural ration. . . . A period of at least 2 years is necessary for the satisfactory assay of the effect of any causative or preventive treatment upon the frequency of caries incidence in the monkeys."

Periodontal Disease

A review of the literature reveals a paucity of reports concerning the nature of diseases of the supporting tissues of monkey teeth (Krishnan, 1933; Williams, 1949). In fact relatively few investigations dealing with experimentally produced periodontal diseases have been reported (Goldman, 1947, 1954, and unpublished data; Ramfjord, 1951; Ziskin *et al.*, 1944). Colyer (1936) in an extensive study concluded that periodontal disease manifestations in monkeys and anthropoids were based on local disruptive processes. According to this

author, monkeys and apes are the only animals that show a significant amount of periodontal disease in the wild state; they state that the incidence increases when the animals are in captivity.

Auskaps and Shaw (1957) studied the dentition of cynomolgus monkeys with respect to the periodontium. They found heavy deposits of calculus, with no particularly destructive tendencies of the periodontal tissues. On the other hand, in another study (Shaw and Auskaps, 1954) on marmosets, periodontal disease was found prevalent, varying from mild gingival involvement to extensive destructive disease that resulted in loss of teeth. The authors suggested that despite the difficulties in maintenance of the marmoset, its incidence of periodontal disease makes it a very useful laboratory animal.

One of us (H.M.G., 1947) reported the findings of a histopathological study of the teeth and supporting structures of a young rhesus monkey that had died after transportation by ship from Africa to the United States. Of interest was the similarity of changes found in this animal to changes reported by various investigators who had placed groups of monkeys on deficient diets. This monkey for six months was on a restricted dietary program that consisted of coarse meal and water, and no bananas or citrus fruits. The animal was one of a large group brought to this country for medical experimentation. The ship captain disclosed that a considerable percentage of the animals had died aboard ship. This particular monkey died the day the ship docked. The post-mortem examination showed that death was due to bronchopneumonia.

The monkey appeared emaciated and dehydrated. The teeth were abraded markedly, which is unusual in so young an animal, and this condition was attributed to the coarse meal in the diet. The gingivae were inflamed throughout, with a necrosis of the interdental papillae that gave them a punched-out appearance (FIGURE 1).

Microscopic examination of histological sections of the jaws revealed a marked attrition of the occlusal and incisal surfaces and flattening of the interdental papillae, with an accumulation of debris over the gingival margin. In most of the interdental areas all the gingivae were composed of inflammatory cells, the margin of which was covered by a necrotic membrane. The gingival fibers were entirely destroyed. The sulcular epithelium and the epithelial attachment were absent. The inflammatory infiltrate extended deeply into the supporting tissues, through the transseptal fibers, and into the marrow spaces of the alveolus (FIGURE 2). There was resorption of the crestal bone.

A comparison of the above findings with those published by various investigators concerning periodontal manifestations in nutritional experiments is of extreme interest. Topping and Fraser (1939) and Fraser and Topping (1942) reported an experiment on the effect of complete ascorbic-acid deficiency in monkeys. Their monkeys developed a generalized necrotic gingivitis, the roots of the teeth having become denuded. There was necrosis of the interdental papillae, and heavy accumulations were to be seen around the teeth. The animals lived an average of ninety-five days.

Chapman and Harris (1941) confirmed the findings of Topping and Fraser (1939). They found that monkeys maintained on certain vitamin-deficient diets developed a tendency to oral lesions accompanied by an increase in the fusospirochetal flora; monkeys maintained on an adequate stock diet did not.

The limited observations of these workers indicated that monkeys maintained on diets deficient in the B complex, except for B_6, and with supplements of vitamins A, D, and C, nicotinic acid, and riboflavin, also tended to develop rather severe oral lesions and marked general symptomatology, and showed short survival times. Day *et al.* (1935) described gingival disease in a folic-acid deficiency.

Shaw *et al.* (1945*b*) produced acute and chronic ascorbic-acid deficiencies in young growing *mulatta* macaques by a ration that was 74 per cent sucrose, 18 per cent casein, 4 per cent salt mixture, 4 per cent cottonseed oil, with added liver concentrate. Adequate supplements of the fat- and water-soluble vita-

FIGURE 1. Upper and lower jaws of a monkey on a deficient diet, with a necrotizing ulcerative gingivitis.

mins were also provided. Of interest were the chronically deprived animals; these were given rations of ascorbic acid just sufficient for maintenance, but still low enough to cause a deficiency state. Gingival changes described by previous investigators were noted (Howitt, 1931; Tomlinson, 1942). In 1949 Shaw again produced a chronic ascorbic-acid deficiency in ten young adult brown capuchin monkeys. The oral lesions were severe, with such destruction of the periodontal tissues that exfoliation of the teeth occurred.

Chapman and Harris (1941) also found that monkeys maintained on an adequate stock diet tended to resist artificial implantation of the fusospirochetal flora under the severest test conditions. The experiment consisted of collecting material from the necrotic gingivae and periodontal tissue of an infected animal with a blunt curette and scalpel and transferring it directly to the traumatized tissue of a healthy animal. Necrotic material was inserted

FIGURE 2. The same monkey as in FIGURE 1. Note the necrotizing ulcerative gingivitis of the interdental area (*top and middle*). Calculus is seen on the gingiva of an erupting molar (*bottom*). Note the underlying break between the oral epithelium and the reduced enamel epithelium.

even into a tooth socket, and no transmission resulted. The wounds healed in normal fashion.

Goldman (1947) described the histopathological changes in the jaws of two spider monkeys that originally had belonged to a group of twenty-five animals used for behavior- and nerve-regeneration experiments. These monkeys were kept in a large enclosure and fed exclusively on waste, fruits, and vegetables. During the experiment they became infected with amoebic and bacillary dysentery. Six that died all showed ulcers of the stomach or intestine, in most cases with perforations. The two jaws studied came from this group. They were examined clinically, radiographically, and microscopically, the clinical observations being supplemented with information concerning the monkeys obtained while they were alive.

One of the symptoms was migration of the teeth, manifesting itself by either extrusion or lateral movement; another was loosening of the teeth.

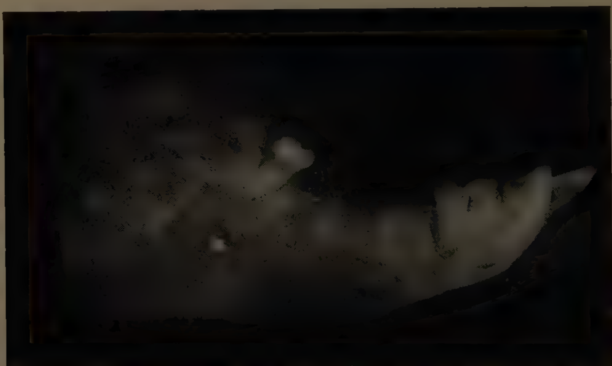

FIGURE 3. X ray of the hemisection of a mandible showing widening of the periodontal ligament space around the last molar and on the distal aspect of the second premolar. The sockets of teeth recently lost may be seen.

Clinical examination of the jaws disclosed varying degrees of pocket formation. There were both firm and loose teeth, some being almost exfoliated. In no instance was there a pocket without some loosening of the tooth. Where there were pockets a slight retraction and rolled margin of the gingiva could be detected; for the most part there was no obvious recession. Calculus formation was abundant and of the soft type. X-ray examination disclosed widening of the periodontal space, some teeth being markedly affected and others not. The widening was localized on one surface of the tooth or encircled the entire tooth root; occasionally in the marginal periodontium it appeared as a conelike radiolucent area. FIGURE 3 shows a marked width of the periodontal membrane. The periodontal ligament showed the chief changes caused by this disease. The early alterations appeared as a loss of the functional structure of the periodontal membrane, the principal fiber apparatus becoming disorganized. The fiber bundles, which under normal conditions are attached from the cementum of the tooth to the bone, appeared to be broken and undergoing degeneration. There was loosening and swelling

of the interfibrillar substance and disintegration of the fibers that finally became loose edematous connective tissue (FIGURE 4). An unusual number of capillaries and edema of the tissue were constant findings. The change could be found localized in relatively small areas of a periodontal ligament and, in other instances, it affected the entire structure.

The disintegration of the principal fibers seemed to arise in the middle portion or the intermediate plexus, the attachment to the cementum and bone persisting for a time. There were old fiber attachments in the cementum even

FIGURE 4. Degenerative changes occurring in the periodontal ligament. Osteoporosis of the supporting bone (*right*) is also evident. Silver stain.

in the late stages of the destruction. These connective tissue changes were of a degenerative character and were not associated with any inflammatory reaction in the periodontal ligament. In the late stages the periodontal ligament consisted of loose hyperemic edematous connective tissue; almost no collagen fibers remained (FIGURE 5).

There was a diffuse osteoporosis, the spongy bone being depleted early. Only a few bone trabeculae occupied the central portion of the alveolus; the marrow spaces were enlarged. Although few areas of osteoclastic activity were noted, irregular indentations on the surface of the bone trabeculae were common. The last condition denoted a resorptive process of the osseous tissue.

Goldman (1954) reported on the observation of dietary protein deprivation

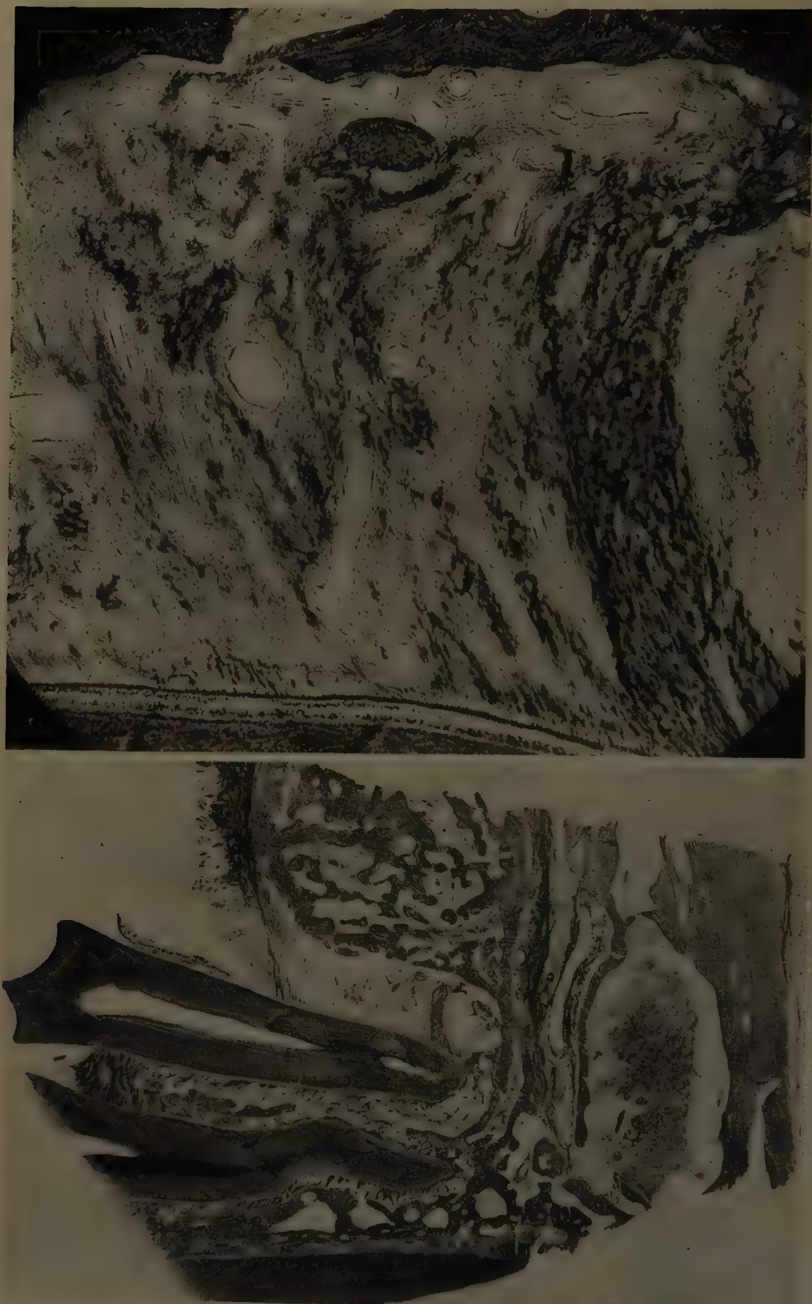

FIGURE 5. *Left*, mandibular premolar showing mesial migration. The radiogram of this specimen is seen in FIGURE 3. Note the persistence of transseptal fibers of the distal of the tooth and some of the fiber apparatus toward the apex. Other than this, the character of the periodontal ligament has been changed. *Right*, high-power photograph of the apical portion of the distal aspect of the tooth. The blotchy character of the collagen of the periodontal ligament is evident.

in juvenile and adult spider monkeys. These animals were divided according to age into two groups of eight. For each experiment, control pairs of monkeys were used. Except for two animals the young monkeys already had shed their deciduous teeth. The adult monkeys had reached full maturity by at least three years.

The diet was 70 per cent starch, 15 per cent nonnutritive fiber, 10 per cent vegetable oil (hydrog.), 4 per cent salt mixture U.S.P. XIV, and 1 per cent cod-liver oil. Vitamin B complex and ascorbic acid were added. This powder

FIGURE 6. Radiograms showing the effects of protein deprivation in two young spider monkeys. *Top*, the widening of the periodontal membrane space around the first and second molars is obvious. There is a mixed dentition. *Bottom*, the widening of the periodontal ligament space is pronounced, and there is a general translucency of the bone.

was mixed with water and formed into round balls that could be picked up by the monkeys. Food and water were fed ad libitum.

Although the animals could not be weighed accurately, their weight loss was evident. Late in the experiment they became lethargic. Seemingly, the young exhibited slight enlargement of the abdominal area. There was some calculus formation and varying degrees of gingivitis occurred in the form of ulcerative necrotizing lesions. In isolated areas deep pocket formation could be detected. The teeth varied in mobility, some being very loose. The radiographically observed changes in the young monkeys were of especial interest in that not only was there distinct diffuse radiolucency throughout

the skull, but characteristic changes were observable in the alveolar and supporting bone. This widening of the periodontal membrane space may be seen in FIGURE 6. Associated with it was a radiopacity of the alveolar bone, which became more distinct. Close examination of this zone of increased density disclosed that it frayed out into the supporting area, where there was a marked translucency. An interpretation of this radiographic feature could not be formed with certainty. The young showed more distinct and exaggerated

FIGURE 7. Mandibular section of a protein-deprived spider monkey showing advanced changes in the supporting tissues. Note the loss of alveolar and supporting bone and degeneration of the periodontal ligament. A necrotizing ulcerative gingivitis is present. Low power.

changes than did the old monkeys. The controls showed no evidence of disturbance.

Histological examination of the jaws revealed changes in the principal fiber apparatus of the periodontal membrane and in the supporting and, to a lesser extent, the alveolar bone (H. M. Goldman, unpublished data, U.S.P.H. Grant D-320). Various stages of alteration were noted. FIGURE 7 shows marked widening of the periodontal membrane space and an osteoporosis of the supporting bone. The collagen fibers in one area became blotchy while, in another region adjacent to it, they were missing. In a later stage an entire section was void of almost all the collagen fibers running from the cementum to the alveolar bone, only short remnants being incorporated in the cementum.

The spongy supporting bone in the earlier manifestations was considerably more affected than the cortical alveolar bone, although the latter became completely altered in the later stages. Active osteoclasis, as in the rat (Goldman, 1954), was not observed. For the most part the bone trabeculae had an etched irregular surface consistent with Howship's lacunae, with no observable osteoblastic layer evident on the opposite side.

Such findings may be communicated best by photomicrographs. These photomicrographs in FIGURE 8 were taken at the same magnification, hence they can be compared for relative size of the periodontal ligament. This ligament, composed of wavy white collagenous connective tissue, is clearly defined in the control; oblique fibers extend from the cementum of the tooth on the left to the bone on the right. The widths of the ligaments should be compared: in the photomicrograph at the right the bone is seen to be undergoing extensive resorption and the fiber apparatus has been modified extensively. FIGURE 9 comprises high-power photomicrographs of the fiber apparatus extending from the tooth in the control (*top*) and in the depleted monkey (*middle and bottom*). Note the absence of collagen fibers in the two lower photographs. FIGURE 10 shows the osteoporosis of the jaws in protein deprivation. FIGURE 11 illustrates the inflammatory lesion in the gingivae. In FIGURE 7 the compact character of the normal alveolar bone is completely disrupted, the tissue being represented by irregularly shaped trabeculae.

Ramfjord (1951) noted a high rate of gingivitis in twenty-one *M. mulatta*; the gingivitis was more severe around deciduous than around permanent teeth. Of interest was his description of the lesions found in the attachment apparatus. Characteristic lesions of occlusal traumatism were evident. These lesions were found in both healthy and systemically affected animals.

One of us (D.W.C.) studied the skulls in the nonhuman primate collection of the Academy of Natural Sciences in Philadelphia for evidence of resorption of the alveolar process. In the thirty-four (gibbon) skulls from the Far East there were no evidences of bone resorption, despite the severe attritional patterns in the teeth of the adult specimens (FIGURE 12). Among the eight siamang skulls there were also attritional patterns, but no carious lesions or osseous resorption. One of the fifteen orangutan skulls showed definite resorptive lesions of the alveolar process. The three adult chimpanzee skulls showed advanced resorptive lesions of the alveolar and supporting bone (FIGURE 13). One of the skulls showed loss of the teeth and evidences of periodontal involvement. With advanced occlusal wear one would expect to observe food impactions because of loss of proximal contacts of the teeth. Hirschfeld (1939) described a chimpanzee picking food from his teeth with a straw. Inquiry regarding this habit revealed that other anthropoid authorities had observed it in various primate colonies. Whether it was an imitation of human behavior, a form of amusement, or an effort to relieve an uncomfortable feeling about the teeth is difficult to determine.

The oral mucous membrane lesions observed in B virus infection in monkeys are significant. The ulcerations involve the tongue, lips, buccal mucosa, and palate and, in many respects, resemble the oral lesions of herpes in man. They usually persist for a period of ten to fourteen days and heal without scarring.

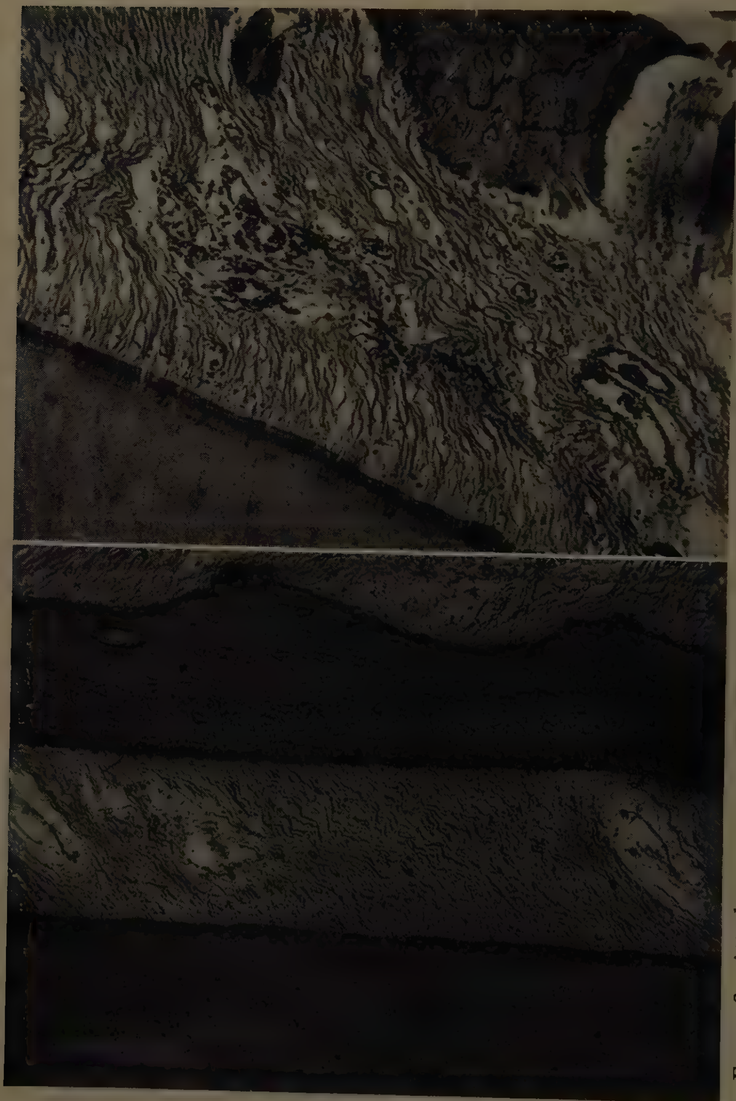

FIGURE 8. Attachment apparatus of a depleted spider monkey (*right*), in contrast to that in the control (*left*). Note the difference in the width and character of the collagen fibers.

A more complete description by Stanley Keeble, along with histopathological correlation, can be found elsewhere in this monograph.

Other viral lesions affecting the salivary glands in primates have been described in the literature (Chu *et al.*, 1951; Cowdry and Scott, 1935*a* and *b*; Vogel and Pinkerton, 1955).

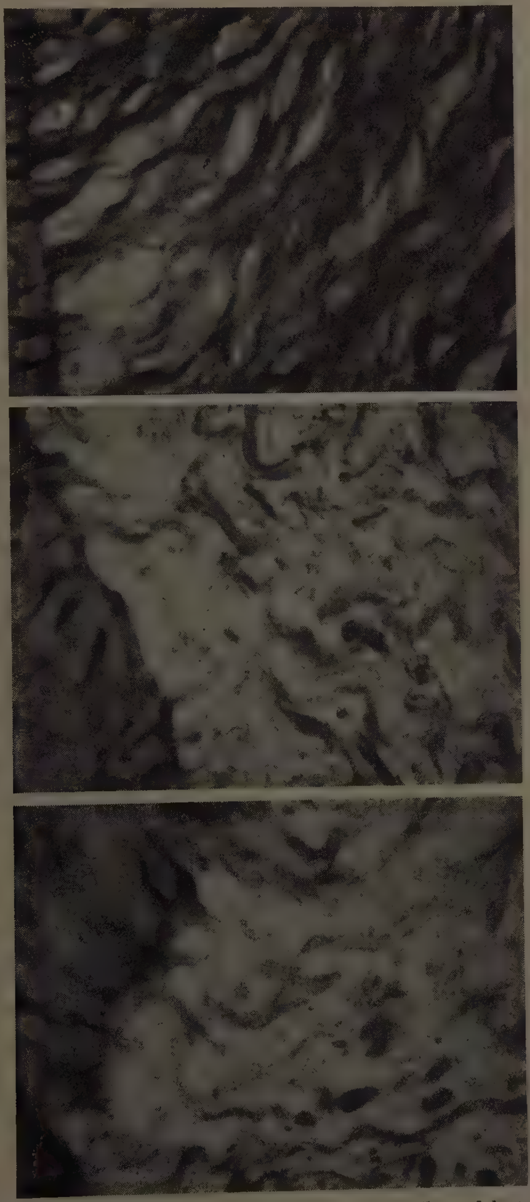

FIGURE 9. *Top*, the collagen fibers of the control. *Middle and bottom*, loss of collagen fibers in protein deficiency.

FIGURE 10. Loss of bone trabeculae in protein deficiency. Marked osteoporosis.

FIGURE 11. Necrotizing ulcerative gingivitis of animals with protein deficiency.

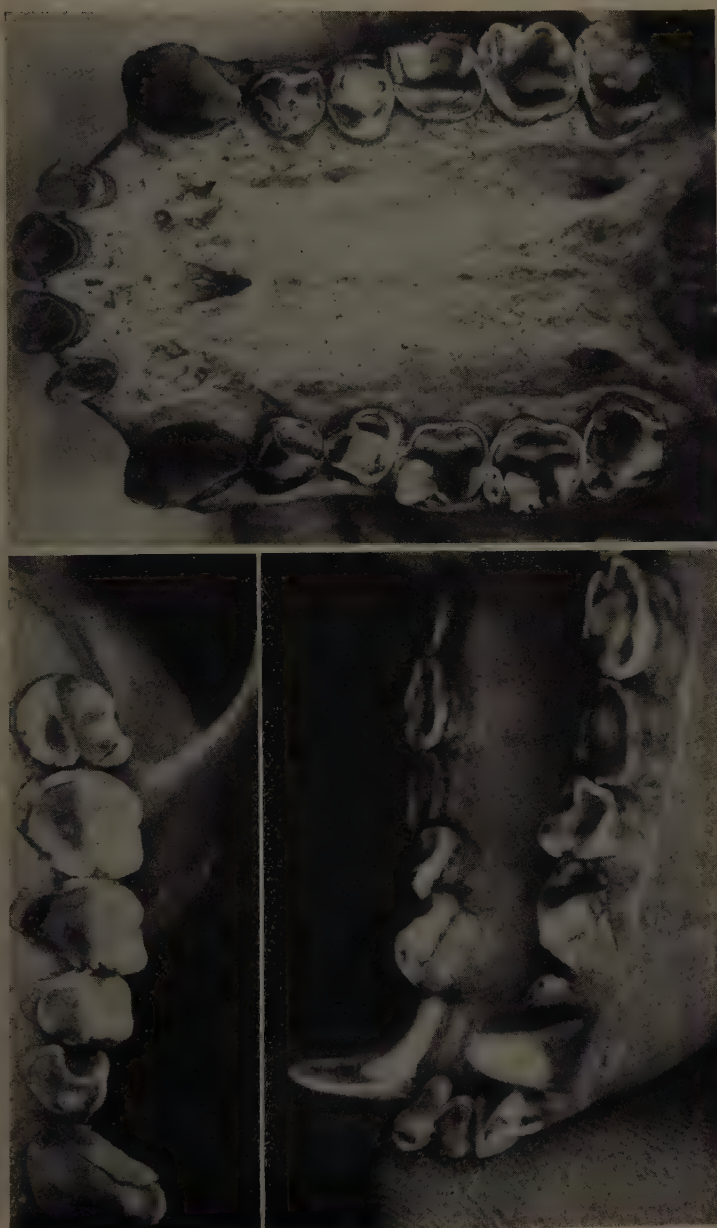

FIGURE 12. *Top left*, attritional wear in the maxilla of a gibbon skull. *Bottom left*, attritional pattern in the mandible of a gibbon skull. *Right*, severe wear in the maxilla of a gibbon skull. Reproduced by permission of The Academy of Natural Sciences of Philadelphia, Philadelphia, Pa.

FIGURE 13. Periodontal disease in two chimpanzee skulls. Alveolar resorption has occurred throughout mouth. Mandibular anterior teeth missing, possibly due to periodontal disease (*upper left*). Alveolar resorption almost uniform throughout the mouth. Note alveolar resorption and also root fenestration in maxillary molar region (*lateral views*). The close-up of the mandibular molar region shows infrabony pocket formation and bifurcation involvement. Reproduced by permission of The Academy of Natural Sciences of Philadelphia, Philadelphia, Pa.

Tumors in the Oral Cavity

A review of the literature discloses the extreme rarity of oral tumors in nonhuman primates. Only 12 cases of neoplasm in the oral cavity have been found in our search of reported cases. The autopsy records in the large zoological gardens, as in Philadelphia and in London, England, reveal that the incidence of neoplastic disease is lower in primates than in any other comparable group of mammals (Hamerton, 1930). The 12 oral lesions constitute 15 per cent of the reported spontaneous neoplasms in nonhuman primates. Interestingly enough, one attempt to transmit a spontaneous growth from the oral cavity of one monkey to another was successful. Steiner *et al.* (1942) and Klüver and Brunschwig (1947) prepared a cell-containing extract from a squamous cell carcinoma of the tongue of a 16-year-old male *M. mulatta* and injected it into the tongue of another male of the same species. Fourteen months later biopsy and histological examination revealed a squamous cell carcinoma of the tongue.

One of the fascinating aspects of this survey of oral neoplasms was that 4 cases, or one third of the total lesions, came from the one colony in which the transplantation was successful. These cases, occurring within a 6-year period, were even more unusual in that malignancies of the same type, carcinoma of the tongue, were found in 3 different species (*M. mulatta*, *M. irus*, and *Saimiri sciurea*). Klüver and his co-workers searched for possible common etiological factors, but were unable to determine any significant environmental causes that supposedly are responsible for oral carcinoma in man. In 2 of these 4 monkeys the carcinoma showed a striking red fluorescence that was due to the ether-soluble porphyrins in the lesions, but their role in the production of disease is uncertain. These animals were being used for neurophysiological and behavioral investigations. Three had undergone brain surgery as part of these studies, and the experimental brain lesions were of long standing when the lingual neoplasms were observed.

Two of the other 8 oral tumors were also in the tongue. Krotkina (1956) reports a squamous cell carcinoma that destroyed the anterior portion of the tongue of a male *mulatta* macaque in the zoo in Leningrad, U.S.S.R. Ruch (1959) cites the unpublished report of Hemmens, who noted a carcinoma of the tongue in a macaque. Hamerton (1930) and Zuckerman (1930) describe a poorly differentiated squamous cell carcinoma on the floor of the mouth of an adult male *M. mulatta* in the London Zoological Garden. A small ulcer was noted just beneath the tongue near the frenulum. This grew rapidly in size and, after 4 months, involved the entire floor of the mouth in front of the tongue and extended onto the gingiva about both canines. Autopsy disclosed that the mandible and salivary glands were infiltrated by the tumor, which also had metastasized to regional lymph nodes. Fox (1934) described a squamous papilloma in the oral pharynx of a *Hylobates lar* in the Philadelphia Zoo.

Kent and Pickering (1958) reported an osteogenic sarcoma of the right maxilla in a three-year-old male *M. mulatta.* The tumor had infiltrated the maxilla and the bones of the skull to the extent that complete excision was impracticable.

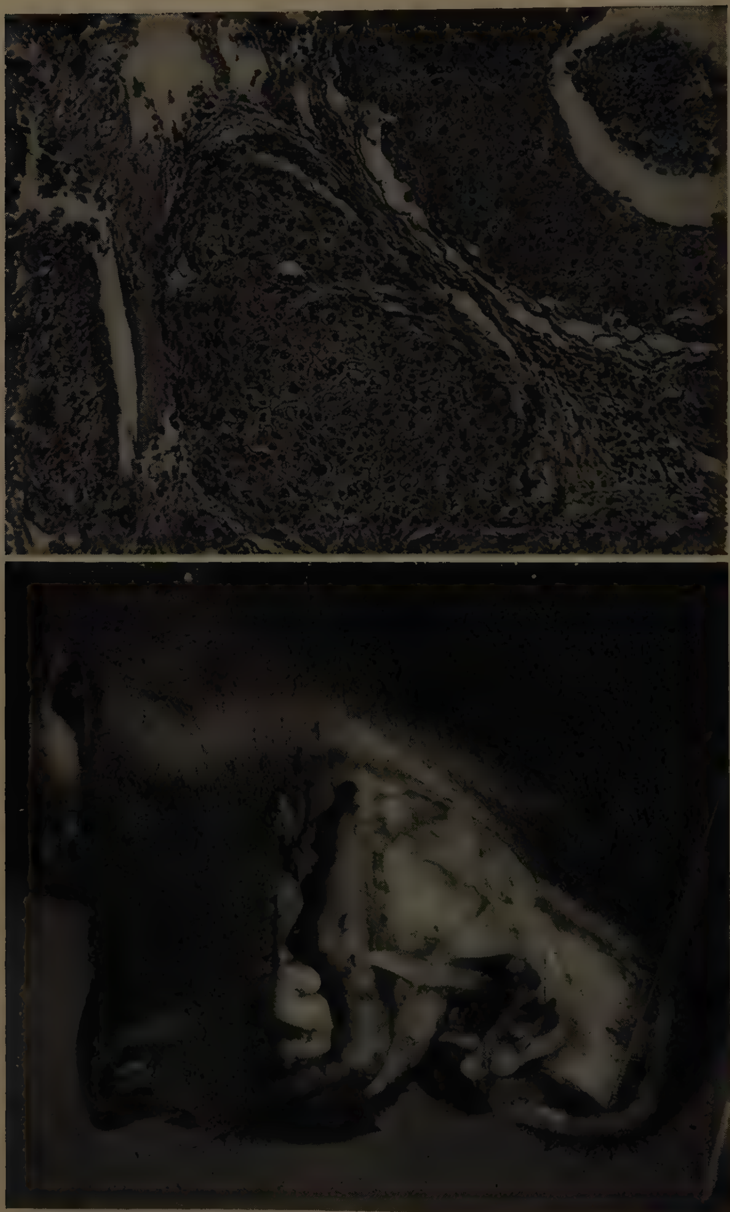

FIGURE 14. *Left*, squamous cell carcinoma of mandibular left gingiva in a baboon. Note displacement of teeth. *Right*, photomicrograph of this lesion.

Bagg (1931) also described an osteogenic sarcoma in the mandible of an adult female *M. mulatta.*

Rankow's case (1947) of a maxillary tumor in a six-year-old male *M. mulatta* has provoked considerable discussion about the possibility that this fibro-osteoma was not a true neoplasm, but a localized manifestation of a systemic metabolic disease:

"Two large symmetrical maxillary protuberances about 6.5 cm. by 2.5 cm. by 5 cm. extended anteriorly and inferiorly, displacing the incisors outward and the canines downward and laterally. The mucous membranes over the growths were intact. When the lesions were sectioned, their contents appeared dirty gray, granular and gritty. Microscopically, there was no calcified cortex, the lesion being encompassed by a fibrous periosteal sac, and many narrow irregular trabeculae were scattered throughout this outer tissue. They were encircled by layers of pink staining osteoid tissue of varying thickness, surrounded by osteoblastic cells. The central portions of these outer trabeculae appeared calcified and deeply stained, being converted into a primitive type of bone. The lacunae were prominent and irregular in arrangement. In contrast, deeper within the lesion normal bone was replaced by tiny areas filled with eosinophilic osteoid tissue. Osteoporosis was indicated by the large vascular spaces in the bony trabeculae. The fatty marrow spaces were completely replaced by dense intermingled strands of collagenous fibers with varying numbers of spindle-shaped nuclei."

Ruch (1959) points out the similarity of this lesion to the condition known as "pseudogeundou," which is always bilateral and favors the maxilla as a site of origin. On the basis of the histological findings, and in the absence of a skeletal survey, Rankow favored the diagnosis of a neoplastic process over that of generalized bone disease.

Schlumberger (1953) and one of us (H.M.G.) examined a baboon at the National Zoological Garden in Washington, D.C., in 1944. The animal had difficulty in eating, appeared listless, and ropy saliva drooled from its mouth (FIGURE 14, *left*). The animal was sacrificed and the autopsy was performed at the Armed Forces Institute of Pathology. An extensive, poorly differentiated squamous cell carcinoma of the mandibular left gingiva was found (FIGURE 14, *right*). The severe destruction of the alveolar process had resulted in the loosening of several teeth. The tumor had invaded the mandible and floor of the mouth and had metastasized to the regional lymph nodes. Gorlin in a personal communication (1959) mentioned that he had observed a complex odontoma in the maxillary incisor area of a chimpanzee. He has also studied a lymphosarcoma in the lower lip of a *M. mulatta*, a fibroma in the upper lip of a baboon, a squamous cell carcinoma of the gingiva of a baboon, and an osteosarcoma in the right maxilla of a monkey.

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EXPERIMENTAL STREPTOCOCCAL INFECTION IN THE RHESUS MONKEY*

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The etiology of rheumatic fever is still an enigma. Although the streptococcus has been implicated through a substantial body of indirect epidemiological and serologic evidence, its specific role in the production of the classic pathological changes associated with rheumatic fever has not been defined clearly thus far. The production of this disease in animals would aid immeasurably in elucidating its etiology, treatment, and prevention. Of prime importance in the development of an experimental model is the utilization of an animal species that is naturally susceptible to pharyngeal infection by group A beta hemolytic streptococci; moreover, a species whose connective tissue response most nearly parallels that of man. The monkey probably meets both of these requirements.

Seegal *et al.*[1] and Pilot[2] reported on the natural occurrence of group A streptococcal infections in monkeys. Boisvert[3] demonstrated that serologic, biochemical, and cultural characteristics of strains of streptococci isolated from monkeys were similar to those of known human pathogens of group A. Watson *et al.*[4] demonstrated, with experimentally induced streptococcal infections in monkeys, clinical and serologic responses that were similar to those of man, and no significant pathological alterations were noted in eleven monkey hearts examined. Eagles and Bradley[5] described "Aschoff-like" nodes near blood vessels in a few monkeys following streptococcal infections. More recently, Hamilton *et al.*,[6] Hamilton and Syverton,[7,8] and Kennedy *et al.*[9] infected monkeys with streptococci by a number of routes. They demonstrated no significant pathological differences from control animals and no alterations typical of those noted in rheumatic fever.

Most of the studies either were not concerned primarily with producing cardiac tissue alterations or else the reports did not give precise descriptions of the alterations. The present study was undertaken because of this relative lack of reported pathological studies of well-controlled streptococcal experiments on the monkey.

This paper reports some preliminary observations made on a series of apparently normal control monkeys and on monkeys subjected to one pharyngeal infection with group A beta hemolytic streptococci. The plan of study was designed to show: (1) whether the histopathological picture of rheumatic fever could be found in a randomly selected control series of animals and the normal spectrum of cardiac alterations in the control series and (2) whether histopathological changes representative of rheumatic fever could be induced in the monkey heart by a single pharyngeal infection with group A streptococci.

* The investigation reported in this paper was supported in part by a United States Public Health Service Fellowship and by Research Grant A-2942 from the National Institute for Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

Materials and Methods

Animals

Ten (5 male and 5 female) tuberculin-free *Macaca mulatta* monkeys weighing from 2 to 3 kg. were used for the experimental study. They were placed on a diet of a commercial monkey biscuit and given water ad libitum.

Organisms

Strains T15/32/3 and T12/30/2* group A beta hemolytic streptococci were used. These were obtained in the lyophilized state from Maclyn McCarty of the Rockefeller Institute, New York, N.Y., and were reported to produce good amounts of M protein. The organisms were grown overnight in 50 cc. of Todd-Hewitt broth, and the resultant culture suspension was centrifuged. The supernatant was removed and the bacteria were resuspended in small amounts of fresh media. Aliquot portions of this preparation then were used for inoculation, titration, and turbidity measurements. Plate and turbidity measurements indicated that the standard inoculum used contained from 2 to 9×10^6 chains of bacteria per cubic centimeter.

Preinoculation Studies

Prior to use in the experiment, all monkeys were isolated individually and studied for a period of from 4 to 6 weeks. During this time each animal's weight and rectal temperature was taken, and white blood counts, including differentials, C-reactive protein determinations, sedimentation rates, electrocardiograms, and phonocardiograms were made biweekly. At weekly intervals, throat, blood, and stool cultures and determinations of streptococcal antibody titers were done. During this control period of observation 2 intradermal eye tuberculin (0.1 OT) tests were done on each animal.

Sedimentation rates were determined by the Westergren method and corrected for hematocrit. C-reactive protein determinations were made by the capillary tube method with commercially prepared antiserum.† The procedure for obtaining the electrocardiograms is reported by Atta and Vanace elsewhere in this monograph. Throat swabs were taken at each examination and placed immediately in sterile tryptose-phosphate broth. A calibrated loopful of this broth medium subsequently was placed in 15 ml. of melted neopeptone agar containing 4 per cent sheep blood, and pour plates were made. These pour plates were incubated at 37° C. overnight and the following morning typical beta hemolytic colonies were counted.

Antistreptococcal serologic tests were done on blood obtained aseptically from the femoral vein. After separation the serum was placed immediately in sterile glass-stoppered tubes and stored at 4° C. Antistreptolysin O and antihyaluronidase titers were determined once weekly according to the methods of Harris and Harris.[10,11] Sera were selected for the determination of antiproteinase titers according to the methods of Ogburn *et al.*[12]

* The number after the first slanted line refers to the number of mouse passages and the final number indicates the number of passages in artificial media after the last mouse passage.

† Specific antiserum obtained from Schieffelin & Co., New York, N.Y.

Monkeys were considered "normal" and acceptable for use in the experiment if they were tuberculin-free, had no significant pathogens in the stools, negative blood cultures, no evidence of beta hemolytic streptococci in repeated throat swabs, corrected sedimentation rates of less than 2 mm./hour, negative C-reactive protein, no rise of streptococcal antibody titers, and no evidence of cardiac murmurs or electrocardiographic abnormalities during the control period of observation.

Infection Procedures

The unanesthetized monkey was restrained in the supine position on a specially adapted restraint board, the jaws were held open with a self-retaining jaw retractor, the tongue was grasped with an Allis clamp, and the pharyngeal region was visualized with a tongue-depressor light source. Two procedures of inoculation were employed. In the first the posterior pharyngeal region was sprayed by means of a small nebulizer with 1 to 2 cc. of the standard inoculum. In the second the standard inoculum was injected into the same region with the use of a long No. 22 needle introduced just beneath the mucosa overlying the tonsillar area bilaterally; approximately 0.5 to 1 cc. of the standard inoculum was injected on each side.

Postinfection Studies

After the inoculation each monkey was placed in its separate cage in an isolation room. All animals in the same room were inoculated with the same strain of streptococcus. Identical studies, carried out according to the preinfection procedures described above, then were made biweekly and weekly. Throat swabs again were taken in duplicate and the pour plates examined for typical beta hemolytic colonies. These were counted and quantitated from 0 to 5+. At least 4 representative colonies from each positive pour plate were picked for extract preparation for grouping and typing according to the methods of Rantz and Randall.[13]

Evidence of Infection

An animal was classified as having an active infection if (1) during the first week following inoculation it demonstrated evidence of pharyngeal infection by at least one positive pharyngeal culture 24 hours or more following inoculation of the same serologic type of streptococcus, (2) there was a significant shift to the left in white blood count (more than 50 per cent polymorphonuclears), sedimentation rate, and C-reactive protein results over control period levels, and (3) there was a significant (at least a 2-tube dilution) rise in levels of 1 or more streptococcal antibodies over control period levels. A subclinical infection was defined as the absence of active infection as determined by the above criteria; the animal, however, showed a significant rise in levels of streptococcal antibodies and serial pharyngeal cultures showing no evidence of interim growth of serologic types of beta streptococci other than the type used for the initial inoculation.

Control Studies

Sham Controls

Two monkeys were subjected to the same preinoculation and postinoculation procedures as was the experimental group except that, instead of streptococcal culture, sterile Todd-Hewitt broth was either sprayed (one monkey) or injected (one monkey) into the posterior pharyngeal region. These monkeys were isolated from the infection group and were sacrificed after four weeks.

"Normal" Controls

Hearts and samples of sera from thirty-six *M. mulatta* monkeys comprised this group. Twenty-five were from established colonies (South Jersey Medical Research Foundation, and Wyeth Institute, Marietta, Pa.) and were sacrificed for kidney cell culture work. Eleven "experimental" control hearts and samples of sera were obtained from monkeys used in live-virus feeding studies at the Wistar Institute, Philadelphia, Pa. None of the eleven animals showed evidence of infection from these tests in the central nervous system and spinal cord histological examinations that followed their sacrifice.

Sacrifice Times

Since the goal of the experiment was the production of cardiac tissue alterations, the monkeys were originally to be sacrificed when clinical or laboratory data indicated that such changes might be present. The majority of the experimental group did not demonstrate these changes and therefore was sacrificed at the time of maximal rise of streptococcal antibody response. Monkey 10 was not sacrificed because the duration of carrier state and antibody response was still to be determined.

Autopsy Studies

Following sacrifice of control and experimental animals at least 7 routine sections of each heart were made for histological study. The areas selected included all valves, both atria and both ventricles, interventricular septum, and auricular appendages. Sections were fixed immediately in 10 per cent cold buffered neutral formalin. At least 6 sections, 5 μ in thickness, were cut from each block. In addition to the routine hematoxylin and eosin stain, the following special and histochemical stains were used: elastic Van Gieson (Verhoeff), periodic acid Schiff (McManus), toluidine blue (Wagner), methyl green pyronine (Movat, Moore), and Pentochrome I (Movat, Moore). Their special techniques are outlined elsewhere.[14,15]

Results

Control Animal Observations

Serologic findings. Figure 1 graphically presents the results of 3 streptococcal antibody titers in 26 to 30 apparently normal monkeys. The antistreptolysin O titers ranged from less than 16 to 256, with a geometric mean value

of 32. The ranges of antihyaluronidase and antiproteinase titers were from less than 16 to 48 and from less than 16 to 384, with geometric mean values of 8 and 32, respectively. In calculating the geometric mean values, titers of less than 16 were treated as values of 8.

Cardiac histopathological alterations. Table 1 summarizes the cardiac alterations in our control series. Eighteen of the 36 hearts demonstrated evidence of myocarditis; 6, periarteritis and 1, valvulitis. In general these alterations were minimal in degree and usually were limited to a focal myocarditis

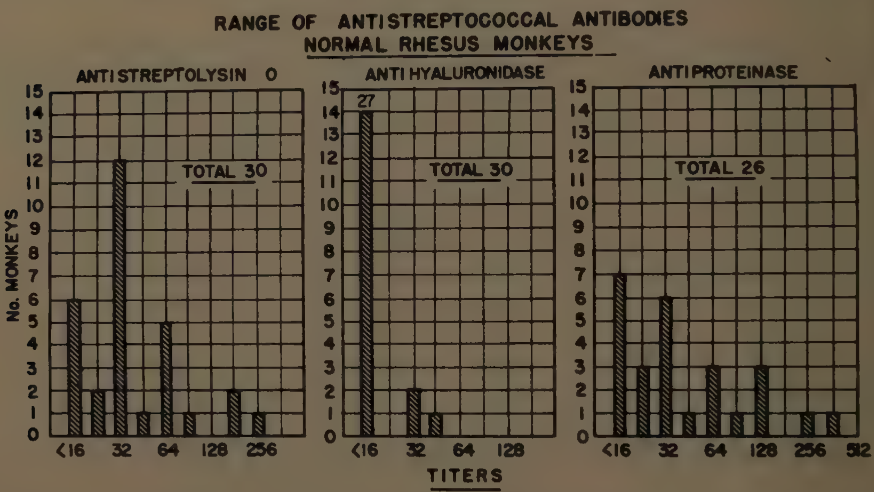

Figure 1. The ranges of three streptococcal antibody titers in 26 to 30 apparently normal rhesus monkeys. The geometric mean values are: antistreptolysin O, 32; antihyaluronidase, 8; antiproteinase, 32.

Table 1
Histopathological Cardiac Alterations in Normal Rhesus Monkeys
At Least 7 Routine Sections of the Heart Were Examined for Each Animal. Any Alteration Noted in at Least One of the 7 Sections Examined was Regarded as a Positive Result

Controls	No.	No. sections examined	Myocarditis* focal	Endocarditis Valvular	Endocarditis Mural	Periarteritis	Pericarditis
Normal†	25	7/monkey	10, +−++ 1, +++−++++	0	0	4 minimal	0
"Experimental"‡	11	7/monkey	6, +−++ 1, +++	1 minimal	0	2 minimal	0
Total	36	252	16, +−++ 2, +++−++++	1 minimal	0	6 minimal	0

* +, Focal myocardial cellular collections with little or no associated myocardial and/or connective tissue alterations. ++, Larger focal myocardial cellular collections plus minimal myocardial and/or connective tissue alterations. +++−++++, Large focal myocardial cellular collections with moderate-to-marked myocardial or connective tissue alterations.

† Monkey hearts obtained from normal animals used for kidney tissue culture work.

‡ Monkey hearts from animals used in live virus feeding studies.

or periarteritis. These 1 to 2+ lesions consisted of focal interstitial collections of small round cells, predominantly of lymphocytes and histocytes. Scattered Anitschkow myocytes and plasma cells occasionally were noted in these lesions (FIGURES 2 to 4). There usually was little or no connective tissue alteration or evidence of myocardial damage associated with these cellular collections. In 2 of the control animals evidence of a more severe myocarditis was noted (FIGURES 5 to 7). One lesion classified as 3+ consisted of a large subepicardial mononuclear cellular collection with minimal muscle damage and connective tissue alteration (FIGURE 5). The 3 to 4+ lesion was larger and exhibited a somewhat different cellular pattern and distinct evidence of muscle necrosis and connective tissue changes (FIGURES 6 and 7). The cellular pattern of this lesion, although basically similar to the smaller 1 to 2+ lesions, contained in addition scattered multinucleated cells and an increased number of cells with intensely pyroninophilic cytoplasm. The associated connective tissue changes consisted of edema, fragmentation of connective tissue fibers, a periodic-acid-Schiff positive and a 1 to 2+ metachromatic ground substance. In no instance, however, did these more extensive lesions resemble Aschoff's bodies. Myocardial lesions in all instances were distributed at random throughout the myocardium, but most were clustered in the ventricular myocardium. They bore no constant relationship to blood vessels. The periarteritis consisted of a perivascular accumulation of small round cells, predominantly lymphocytes and histocytes. The adventitia alone was involved (FIGURE 8). Endocarditis and, particularly, valvulitis were rarely encountered in the group. In 1 animal a moderate mononuclear cellular infiltration was noted in the region of the mitral annulus and adjacent myocardium. No significant alterations of the surrounding myocardium or connective tissue were noted (FIGURE 9).

Experimental Animal Results

Clinical and Laboratory Findings During Control Observation Period

During this period of from 4 to 6 weeks laboratory examinations were made to establish norms for the group and specifically for each animal prior to infection.

Rectal Temperatures

These showed no constant pattern The daily average temperature after approximately 10 min. of rest was 102° F. Variations of ±1.5° F. were not considered significant.

White Blood Counts

The counts of the group as a whole ranged from 10,000 to 22,000/cu. mm. Serial counts made at biweekly and weekly intervals on individual monkeys did not vary more than ±5000. Differential white counts of the group and serial determinations of the same animal fell within a fairly narrow range. The usual pattern was one of a definite lymphocytosis of 70 to 80 per cent, 15 to 30 per cent segmented neutrophils, 2 per cent band forms, 1 per cent juveniles, 2 per cent monocytes, 1 to 3 per cent eosinophils, and an occasional basophil.

FIGURE 2. Microscopic section of the myocardium of a normal control rhesus monkey. Typical 1+ lesion. Note the focal collection of small lymphocytes and mononuclear cells and the absence of myocardial and connective tissue alterations. Hematoxylin and eosin; ×400; slightly reduced from original.

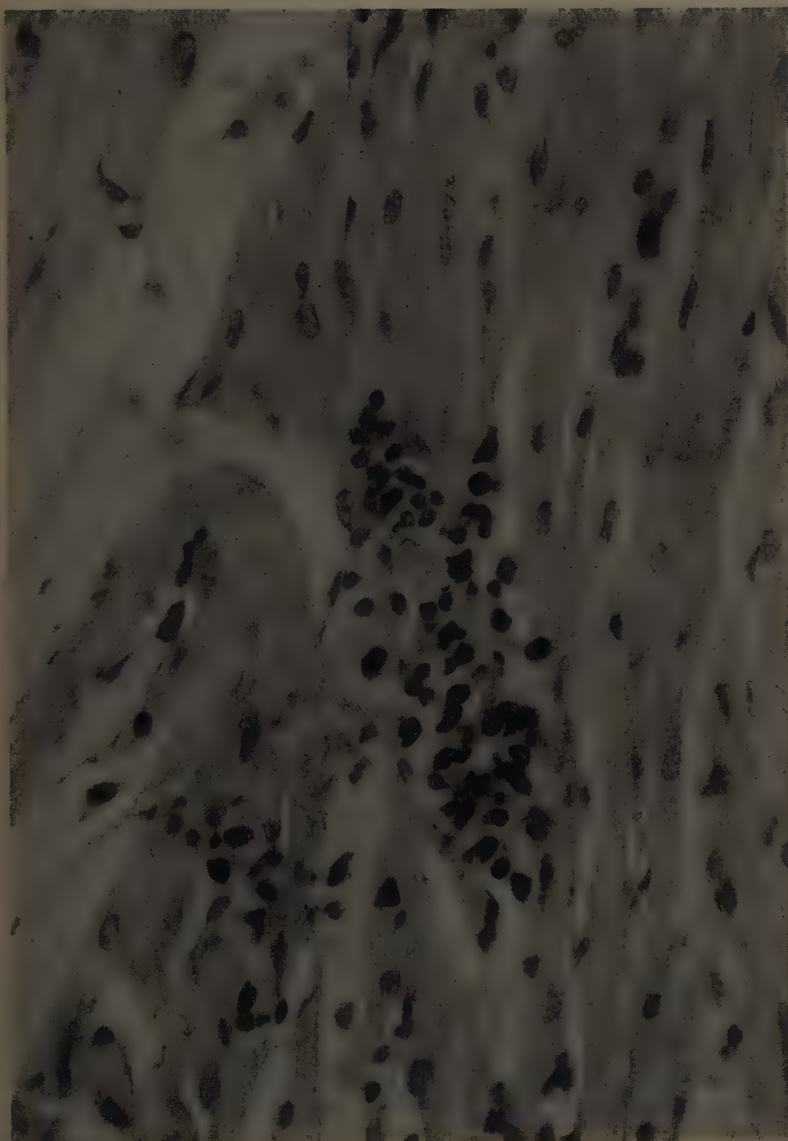

FIGURE 3. Microscopic section of a myocardial lesion similar to that of FIGURE 2 in a normal control animal. A 1+ lesion made up of a focal collection of mononuclear cells with no evidence of myocardial or connective tissue change. A few cells in this lesion (*lower left*) demonstrated great cytoplasmic pyroninophilia. Methyl green pyronine; ×400; slightly reduced from original.

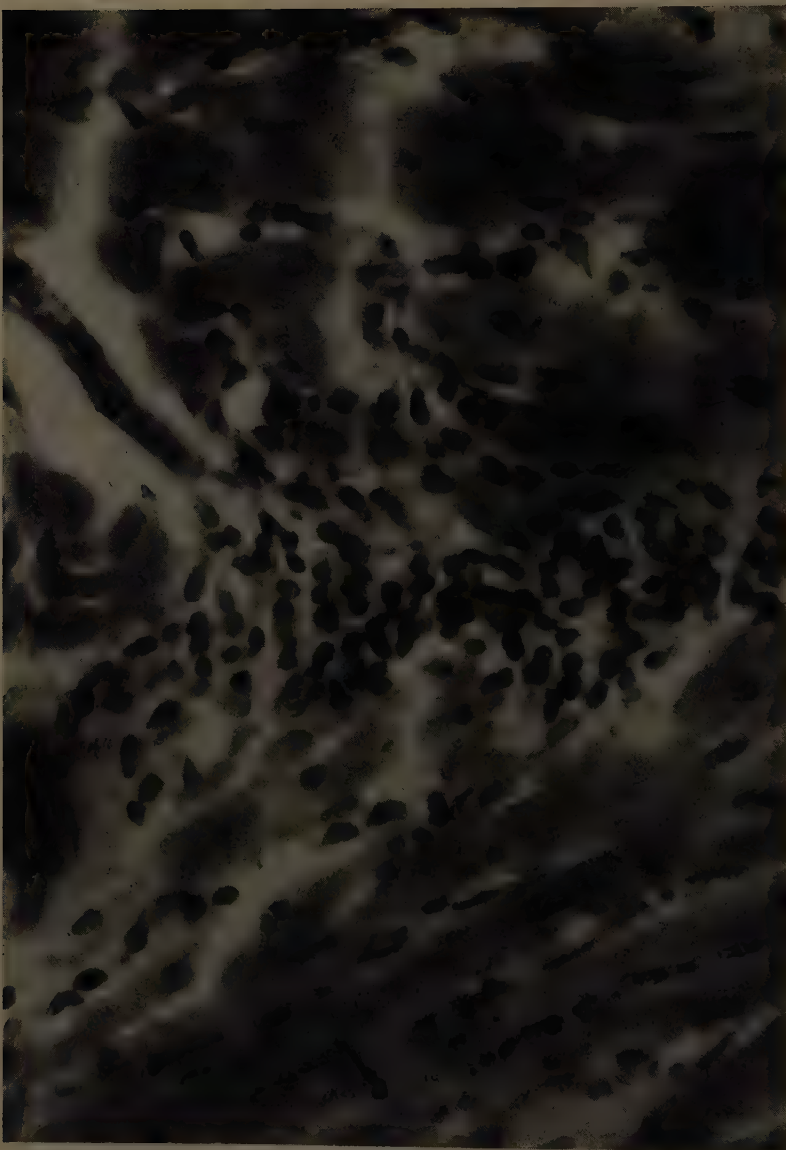

FIGURE 4. Microscopic section of the myocardium of a normal control rhesus monkey. A typical 2+ lesion made up of a focal collection of lymphocytes, histocytes, and scattered Anitschkow myocytes. Early myocardial damage and connective tissue alterations are evident. Hematoxylin and eosin; ×400; slightly reduced from original.

FIGURE 5. Microscopic section of a 3+ lesion in the subepicardium of a normal control rhesus monkey. Note the rather extensive mononuclear cell collection associated with minimal muscle damage and connective tissue alteration. Hematoxylin and eosin; ×100; slightly reduced from original.

FIGURE 6. Microscopic section of the myocardium of a normal control rhesus monkey. A 3 to 4+ myocardial lesion demonstrating a more severe myocarditis with a marked increase of mononuclear cells and further myocardial damage and connective tissue alterations. Hematoxylin and eosin; ×100; slightly reduced from original.

FIGURE 7. An area in FIGURE 6. Higher power. Note the many necrotic myocardial cells and the edema and fragmentation of the connective tissue fibers. The ground substance in and surrounding this focal area of myocarditis was PAS-positive and metachromatic. Hematoxylin and eosin; ×400; slightly reduced from original.

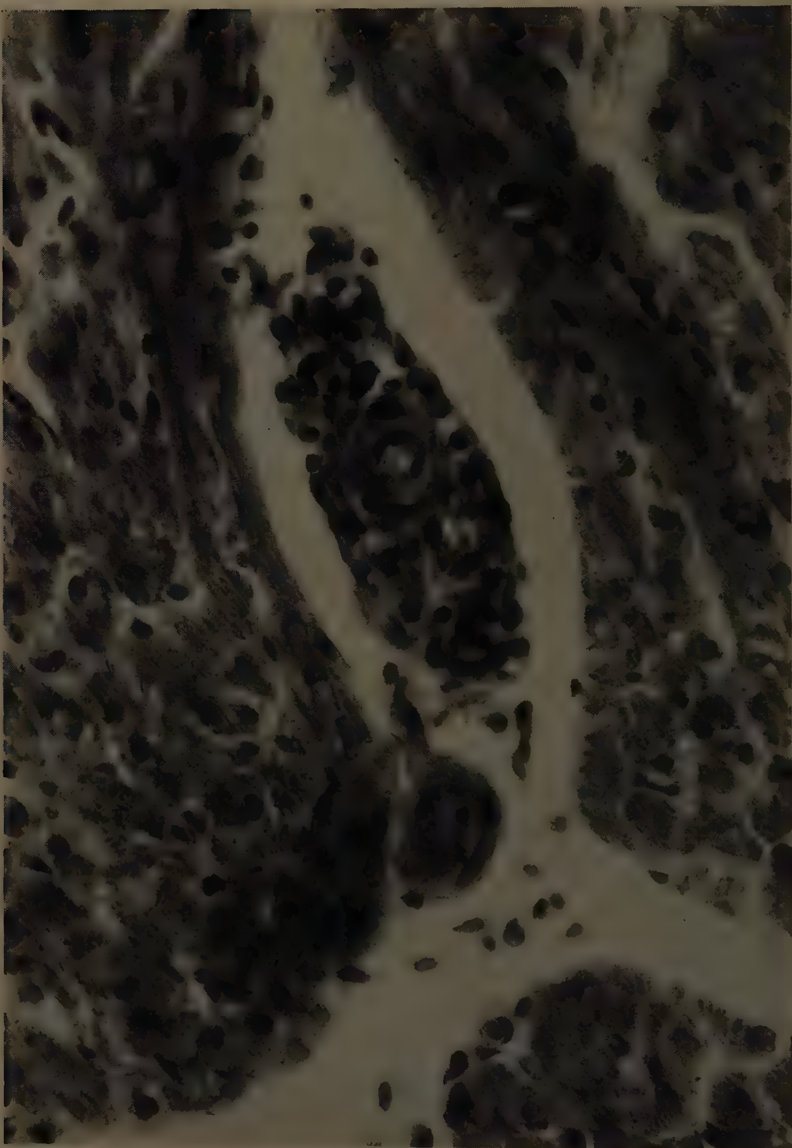

FIGURE 8. Microscopic section of a small myocardial blood vessel in a normal control rhesus monkey. There is a perivascular accumulation of mononuclear cells in the adventitial stroma. Note the absence of muscular and intimal involvement of the vessel. Hematoxylin and eosin; ×400; slightly reduced from original.

FIGURE 9. Microscopic section of the mitral valve in a normal control rhesus monkey. Note the collection of small mononuclear cells in the region of the annulus, extending into the adjacent myocardium. Hematoxylin and eosin; $\times 100$; slightly reduced from original.

A consistent shift to the left of more than 50 per cent segmented neutrophils, associated with an increase of band forms was considered abnormal. None of these animals demonstrated such a change during the control period of observation.

Sedimentation Rates and C-Reactive Protein

All sedimentation rates of control animals were less than 2 mm./hour. C-reactive protein determinations on all animals were negative during this period.

Streptococcal Antibody Titers

The majority of these animals initially demonstrated antistreptolysin O and antihyaluronidase titers of less than 32 and no increase during the control period. Two monkeys had initial antistreptolysin O titers of 128 and 256, respectively, but antihyaluronidase titers of less than 32; the serial antistreptolysin O titers fell to values of less than 32 during the control period.

Cultures

Serial stool cultures for enteric pathogens on all animals were negative, serial blood cultures were negative, and throat cultures for beta hemolytic streptococci consistently negative.

Electrocardiograms and Phonocardiograms

From each animal an average of six phonocardiograms and electrocardiograms was made during this period. There was no evidence of any significant change from previously established normal values (*see* Atta and Vanace, elsewhere in this monograph).

Clinical and Laboratory Findings Following Inoculation

None of the monkeys except monkey 10 showed clinical signs of illness following inoculation. Monkey 10 had an erythematous rash over the face and abdomen 3 days after inoculation that faded rapidly without desquamation during the next 24 hours.

TABLE 2 summarizes the laboratory findings in the experimental group following exposure. Monkeys 1 to 9 and the sham controls showed no significant differences from control levels in rectal temperatures, white blood counts and differentials, sedimentation rates, or C-reactive protein determinations. Stool and blood cultures from all animals were negative. The average duration of positive throat cultures of beta streptococci in monkeys 1 to 8 was 5 to 6 days. Quantitatively, the pour plate results after inoculation in monkeys 1 to 8 were 4 to 5+ after the first 24 hours, and subsequently gradually fell to zero during the first week or 10 days. Monkey 10 (FIGURE 10) demonstrated a significant temperature rise, elevated sedimentation rate, and positive C-reactive protein and was classified as having an active infection. Monkeys 9 and 10 (injection technique animals) yielded positive throat cultures for beta streptococci for 4 weeks and 8 months, respectively. From monkey 9 a plate count of 4+ was obtained for 3 weeks; it fell to 2+ at 4

TABLE 2

SUMMARY OF LABORATORY AND HISTOPATHOLOGICAL FINDINGS IN THE EXPERIMENTAL RHESUS MONKEYS AFTER EXPOSURE TO STREPTOCOCCUS

Monkey no.	Strep. type	Method infection	Infection response	Laboratory*			Antibody response†					Culture		ECG	Sacrif. time after infect. (weeks)	Pathological change‡
				WBC	SR	CRP	ASO	AH	AP	Begin (days)	Max. (weeks)	Throat (days)	Blood			
1	15	Spray	Subclin.	0	0	0	++	0	+++	11	3	9	0	0	3	+ focal myocard.
2	15	Spray	—	0	0	0	0	0	0	—	—	1	0	0	3	+ focal myocard.
3	15	Spray	Subclin.	0	0	0	+++	0	0	14	3	6	0	0	3	—
4	15	Spray	—	0	0	0	0	0	0	—	—	2	0	0	3	—
5	15	Spray	Subclin.	0	0	0	+++	0	—	10	3	10	0	0	6	+ focal myocard. + periarter.
6	12	Spray	Subclin.	0	0	0	+++	0	—	14	4	8	0	0	4	—
7	12	Spray	—	0	0	0	0	0	—	—	—	1	0	0	4	++ focal myocard.
8	12	Spray	Subclin.	0	0	0	++	0	—	11	3	5	0	0	3	—
9	12	Inject.	Subclin.	0	0	0	++	0	++	8	3½	4 wk.	0	0	4	—
10	12	Inject.	Active	+	+	+	+++++	++	++	6	2	8 mo.	0	0	—	—
C1	Media	Spray	—	0	0	0	0	0	—	—	—	Neg.	0	0	4	—
C2	Media	Inject.	—	0	0	0	0	0	—	—	—	Neg.	0	0	4	+ focal myocard.
Total and averages	5-T15 5-T12 2-C	8 spray 2 inject.	1 active 6 subclin.	1	1	1	7	1	3	Av. 10+	Av. 3	Av. 5+ days not incl. 9 & 10	0	0	Av. 4	5 of 11 animals

* +, An increase of over 50% segmented neutrophils with an increase of band cells. SR (sedimentation rate): +, a significant increase over control levels, i.e., greater than 5 mm./hour. CRP (C-reactive protein): +, any evidence of capillary tube precipitation.

† +, At least a 2-tube dilution rise, i.e., ++ = 2-tube dilution rise, etc. ASO, antistreptolysin O; AH, antihyaluronidase; AP, antiproteinase.

‡ Indicates quantitative alterations in at least 1 of 7 cardiac sections examined.

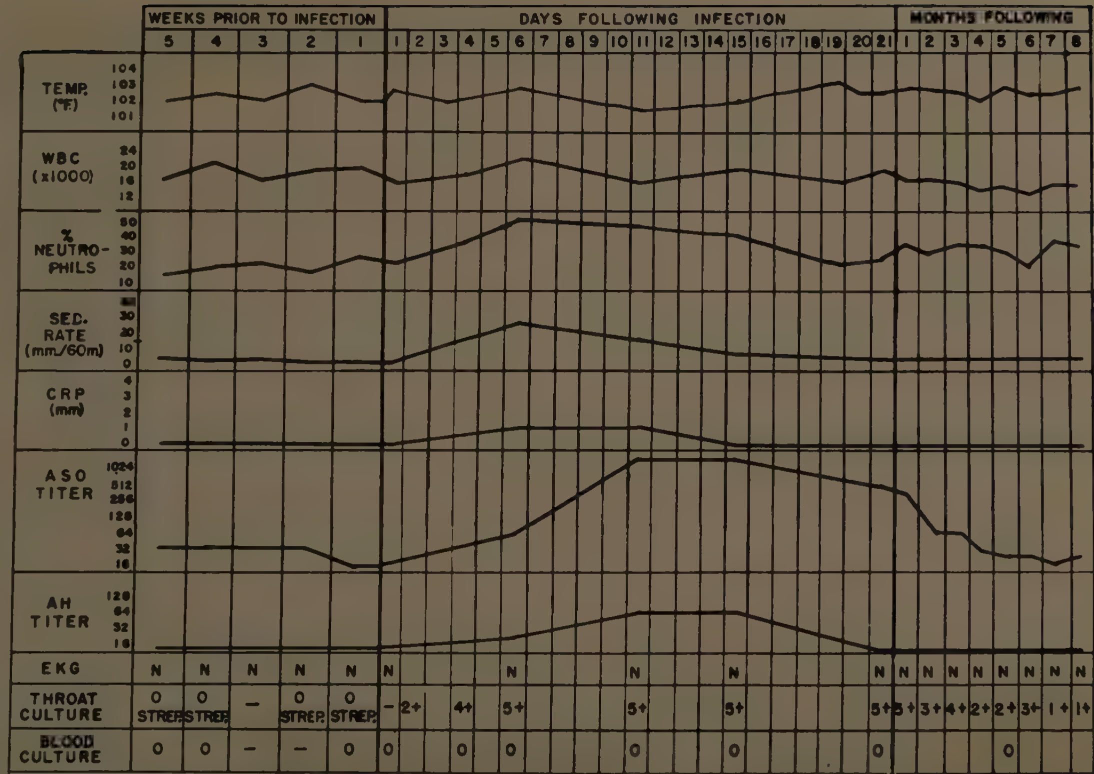

FIGURE 10. Summary of the laboratory findings in monkey 10 before and after exposure to type 12 streptococcus. Note the definite shift to the left in the differential white blood count (WBC), the elevated sedimentation rate, and the appearance of a positive C-reactive protein (CRP) following exposure. The times of beginning rise, maximal titer, and duration of antistreptolysin O (ASO) and antihyaluronidase (AH), responses are indicated. Note also the 8 months' duration of positive throat cultures for type 12 streptococcus.

weeks, when the animal was sacrificed. For 6 months monkey 10 consistently had a count of 4 to 5+ that gradually fell to 0 over the next 2 months.

From each positive pour plate 4 representative beta hemolytic colonies, giving a total of over 150 determinations, were selected for grouping and typing. In all instances grouping and typing revealed identical organisms from each plate and were identical with the organisms originally inoculated in each case.

Seven of 10 inoculated animals demonstrated a significant (at least a 2-tube dilution) rise in one or more streptococcal antibody titers, indicating a subclinical infection response in 6 of these animals. Monkey 10 was the only animal demonstrating a rise in antihyaluronidase titer. The average times of initial and maximal rises of antibody response were 10 days and 3 weeks, respectively. The duration of antibody response and the time of return to preinoculation levels were followed only in monkey 10 (FIGURE 10).

None of the animals demonstrated any significant electrocardiographic or phonocardiographic changes.

The alterations noted by microscopy in the hearts of the experimental and sham control groups were quantitatively and qualitatively similar to those in the control series. All lesions were 1 to 2+ myocardial foci; there was an occasional perivascular accumulation of cells. There were no, or minimal, myocardial or connective tissue changes associated with these lesions and none suggested Aschoff's bodies. There were no instances of valvulitis or pericarditis.

Discussion

The studies herein reported were preliminary attempts at evaluating the cardiac alterations in the normal monkey and assaying the effect on the monkey heart of a single pharyngeal infection with group A beta hemolytic streptococci.

The quantitative and qualitative analysis of spontaneously occurring cardiac lesions in the monkey was a prerequisite of this study. If the supposition that infection with group A hemolytic streptococci in a primate can lead to the pathological counterpart of rheumatic fever is true, it should be possible to find the histological hallmark of this disease in a certain percentage of routine autopsies on the animal. To my knowledge, true Aschoff bodies in monkey hearts have not been described. Hamilton and Syverton[8] examined the hearts of 216 apparently normal monkeys. They found evidence of carditis in 21 per cent of 38 control animals, 66 per cent of 75 animals subjected to mock inoculation procedures, and 80 per cent of 46 monkeys inoculated with poliomyelitis or Coxsackie virus. No detailed descriptions of these changes were made, but no change resembled those associated with rheumatic fever in man.

The incidence of spontaneously occurring lesions in our control series is similar to those of the previous studies. The alterations noted in the present study were usually minimal in character and associated with little or no myocardial or connective tissue alterations. Similar lesions have been reported to occur spontaneously (Miller[16] and Loewe and Lenke[17]) in rabbits and have been noted in previous studies of swine (Vanace *et al.*[18]). Those few naturally occurring lesions that were more extensive and were associated with myocardial

necrosis, fragmentation of connective tissue fibers, and changes in the ground substance could not be termed true Aschoff's bodies. The determination of the cause of these lesions in the control group was not within the scope of this study. No cultures of the cardiac tissues were made. However, no evidence of viral inclusion bodies or bacteria was given by appropriately stained sections. In this connection, Cheever and Heberling (see their report elsewhere in this monograph) found no evidence of viral agents in tissue cultures from 48 apparently normal monkey hearts.

Although the number of animals in the experimental group was not adequate for a statistical analysis, a few observations are worth considering. Both active and subclinical streptococcal infections could be elicited in the monkeys with the techniques described. The serologic response of streptococcal antibodies that followed these infections agreed with the conclusions of other workers[4] that the response following streptococcal infection was, in the monkey, similar to that in man. The production of an active infection in one monkey and the persistence of the streptococci in the pharynx in two animals for periods of four weeks and eight months, respectively, may indicate that the injection technique is the future method of choice.

Since the incidence of cardiac alterations in the experimental group was similar quantitatively and qualitatively to that in the control, the results could not be ascribed to the experimental procedure. These results are in agreement with the work of Kennedy *et al.*,[9] who noted myocardial lesions in 7 of 8 monkeys after repeated introductions of virulent streptococci into the dental root canals. The lesions, however, were similar to those noted in 3 of 4 sham control animals and were not comparable to rheumatic fever lesions. Hamilton *et al.*[6] and Hamilton and Syverton[7] noted changes suggestive of rheumatic disease and Aschoff-like nodules in some monkey hearts after repeat injections of streptococci inactivated *in vitro* by penicillin, alone and in combination with foreign protein. Later, Hamilton and Syverton[8] reported an incidence of 85 per cent carditis in 57 monkeys inoculated with streptococci and/or foreign proteins. Unfortunately, adequate descriptions of these lesions and a critical comparison with the naturally occurring lesions in the control normal monkeys were not presented.

This work is being continued along a number of lines. A more complete study of a larger control series is in progress in order to more clearly define the naturally occurring lesions in the monkey and in the hope of finding the naturally occurring histological counterpart of rheumatic fever in this species. The direct and indirect streptococcal bactericidal test described by Rothbard[19] and Lancefield[20] will be utilized on control and experimental blood for a further knowledge of type-specific immunity in these animals. Repeat serial pharyngeal infections with different serologic types of streptococci and various extracellular products of streptococci in hypersensitivity experiments are in progress. Additional tests, such as serum transaminase and glycoprotein determinations, are being made to determine more precisely the times that cardiac tissue damage is most likely to be present and when the animals are to be sacrificed.

It is hoped that the final evaluation of all this work will provide a better

understanding of the relationship of the streptococcus to the production of the cardiac alterations noted in rheumatic fever in man.

Summary

This report presents preliminary observations on attempts to produce in the rhesus monkey (*Macaca mulatta*) the pathological counterpart of rheumatic fever by means of one pharyngeal infection with group A beta hemolytic streptococci. Prior to the experiment a pilot study was made of the normal spectrum of streptococcal antibody titers and cardiac histopathological alterations occurring in a control group of thirty-six rhesus monkeys.

Serum levels of three streptococcal antibodies in this control group demonstrated that this species of animal was quite susceptible to infections with the streptococcus. The incidence of spontaneously occurring myocardial lesions in this control group was 50 per cent. The majority of these lesions was merely focal interstitial collections of small round cells with little or no associated myocardial or connective tissue changes and they resembled naturally occurring myocardial lesions noted in other species of animals. The few more extensive lesions encountered, although associated with varying degrees of myocardial and connective tissue alterations, did not resemble Aschoff's bodies.

The experiment was carried out on ten normal rhesus monkeys. During a suitable control period of observation normal values for a number of acute phase reactants were determined. Five animals were inoculated with type 15 streptococci and 5 with type 12. These animals plus two sham controls were then followed at regular intervals by means of a number of laboratory tests for evidence of infection and cardiac tissue damage. At specified intervals an animal was sacrificed and sections of the heart were examined with a number of special stains for evidence of tissue alterations.

Analysis of the experimental data indicated that following inoculation:

One monkey sustained an active and six monkeys a subclinical infection.

Seven monkeys demonstrated at least a two-tube dilution rise over control levels of one or more antistreptococcal titers.

The antibody response to streptococcal infection with respect to time sequence, beginning rise, maximal level, and duration of response, was similar to that of man.

One animal (not sacrificed) developed a pharyngeal carrier state of the injected streptococcus of 8 months' duration.

Results of the cardiac tissue studies demonstrated no quantitative or qualitative differences from the control normal monkeys. No Aschoff's bodies were produced.

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ANIMAL INFECTIVITY OF AEROSOLS OF MONKEY B VIRUS

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The epidemiology of the disease caused by monkey B virus has not been clearly defined either in monkeys or in man. A number of cases in humans has been reported in which the virus apparently gained entry through monkey bites. There are fatal cases described in the literature in which the investigators suggest such other routes of entry as the nose, mouth, and eye.

In order to obtain information on the possible role of aerosols in the transmission of monkey B virus, the American Pharmaceutical Manufacturers Association made a request to the Chief Chemical Officer, United States Army Chemical Corps, that some aerosol studies be carried out at Fort Detrick. Following is a summary of the information obtained from exposing monkeys, rabbits, guinea pigs, rats, and mice to aerosols of monkey B virus.

Virus

The virus used in these studies was the fourth rabbit-kidney tissue-culture passage of the O'Hara strain of monkey B virus isolated from a human being whose case was fatal.* When assayed in primary cultures of rabbit kidney and by subcutaneous injection of rabbits, the virus pool titered $10^{8.2}$ TCD_{50}/ml. and $10^{7.9}$ LD_{50}/ml., respectively.

Animal Exposures

Animals were exposed for 5 min. in the Model 3 Henderson aerosol apparatus to clouds generated by the Collison atomizer (FIGURE 1). During exposures 1-min. cloud samples were taken by means of the Porton impinger containing equal quantities of medium 199 and calf serum. Impinger samples were assayed in 4-day-old tissue cultures of primary rabbit kidney in order to determine cloud concentrations of virus.

The amounts of virus inhaled by the various species of animals are presented in TABLE 1. The inhaled dose, expressed as TCD_{50}, is calculated by multiplying cloud concentration (TCD_{50} per liter) by respiratory volume (liters per min.) by exposure time (min.). The amount of virus that is actually retained in the lungs of animals may be only 10 to 20 per cent of the calculated inhaled dose.

Results

In TABLE 1 the italicized figures indicate those doses that induced lethal infections in rabbits, monkeys, and guinea pigs. Rabbits that inhaled from 100 to 30,000 TCD_{50} of virus died. Deaths of monkeys and guinea pigs also occurred, but only at the higher doses. None of the rats or mice exposed to B virus died during the 30-day observation period; however, 2 rats exhibited signs of infection.

* Produced and supplied by Merck Sharp & Dohme, Philadelphia, Pa., as a tissue-culture product.

TABLE 2 shows the number of rabbits that died at the 4-dose levels of virus tested. Mortality ranged from 11 per cent at the lowest dose to 100 per cent at the highest. Deaths occurred from 4 to 10 days after exposure. For about

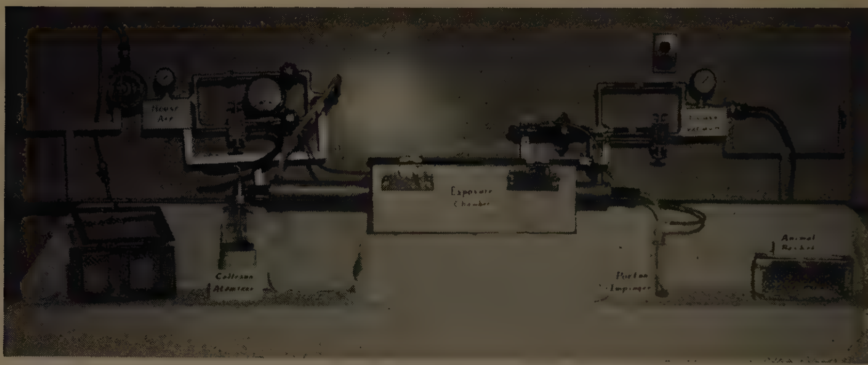

FIGURE 1. Model 3 Henderson aerosol apparatus.

TABLE 1
EXPOSURE OF FIVE SPECIES OF ANIMALS TO AEROSOLS OF MONKEY B VIRUS

Animal	Amount of B virus inhaled (TCD_{50})			
	A	B	C	D
Rabbits	*100**	*400*	*6,300*	*30,000*
Monkeys	250	*13,000*	25,000	*50,000*
Guinea pigs	10	100	*1,200*	*10,000*
Rats	3	25	400	3,000
Mice	2	15	250	2,000

* One or more deaths of animals receiving doses, italicized.

TABLE 2
RABBIT MORTALITY CAUSED BY EXPOSURE TO AEROSOLS OF MONKEY B VIRUS

Inhaled dose of B virus (TCD_{50})	Dead/exposed	Mortality (%)	Day of death after exposure
100	1/9	11	5
400	4/9	44	5, 7, 7, 10
6,300	8/9	89	4, 5, 5, 6, 6, 6, 6, 7
30,000	9/9	100	4, 4, 4, 5, 5, 5, 5, 5, 6

48 hours before death the following signs of infection usually were observed: hyperesthesia, torticollis, labored breathing, salivation, ocular and nasal discharge, conjunctivitis, and corneal opacity. No paralysis was observed. The only other gross abnormal condition observed at post-mortem examination was lung consolidation. The presence of B virus in rabbits that died from aerosol infection was determined by inoculating normal rabbits with suspensions of

lung, brain, and conjunctiva. A typical B virus ascending paralysis developed and death of these rabbits occurred 7 to 12 days after inoculation.

TABLE 3 summarizes the responses of 6 monkeys (*Macaca mulatta*) that were exposed to aerosols of B virus.

Monkeys 721, 713, 82, and 3, which had inhaled from 250 to 25,000 TCD_{50} of B virus, showed no signs of illness during the 30-day period following exposure. Sera collected on the thirtieth day after exposure gave evidence of a twofold and eightfold increase in neutralizing antibody in monkeys 721 and 713, respectively. Sera collected from all 6 monkeys before exposure were diluted and tested against 80 TCD_{50} of B virus. Antibody titers ranging from <1:4 to >1:16 are shown in TABLE 3.

Monkey 732 died on the eighth day after inhaling 50,000 TCD_{50} of B virus. The following signs of illness were observed about 24 hours before death: inactivity, loss of appetite, 104.6° F. temperature, and respiration rate −113. The

TABLE 3

RESPONSE OF RHESUS MONKEYS EXPOSED TO AEROSOLS OF B VIRUS

Monkey No.	Amount of B virus inhaled (TCD_{50})	Neutralizing antibody titer*		Day of death after exposure
		Pre-exposure	Postexposure	
721	250	1:4	1:8	
713	13,000	1:4	1:32	
82	13,000	1:4	ND†	
25	13,000	<1:4	ND	5
3	25,000	>1:16	ND	
732	50,000	1:4	ND	8

* Dilution of serum which neutralized 80 TCD_{50} of B virus.
† Not determined.

only gross lesion observed at post-mortem examination was extensive lung consolidation.

Monkey 25, which had no demonstrable neutralizing antibody before exposure, died 5 days after inhaling 13,000 TCD_{50} of B virus. Death occurred without any signs of illness except an increased rate of respiration and a subnormal temperature. The only gross lesion observed at post-mortem examination was lung consolidation; edema fluid was present.

Bacteriological examination of 10 tissues and blood from monkey 25 revealed beta-hemolytic staphylococci in the lungs. The concentration of bacteria was about 1400 organisms/ml. of 10 per cent lung suspension. This staphylococcus liquefied gelatin, fermented mannitol, and was coagulase-positive; however, it was not lethal for a monkey that was exposed to a large aerosol dose.

Suspensions of tissues and blood from monkey 25 were assayed in rabbit-kidney tissue cultures and also by injection of rabbits, for isolation of B virus. The following monkey tissues produced cytopathogenesis in epithelial cells of rabbit kidney: lung, salivary gland, spleen, axillary lymph node, spinal cord, and buccal mucosa. The concentration of virus recovered from lung material was about $10^{4.3}$ TCD_{50}/ml. of 10 per cent suspension.

Paralysis and death occurred in rabbits inoculated with suspensions of the

following monkey tissues: lung, salivary gland, spleen, axillary lymph node, spinal cord, buccal mucosa, brain, liver, kidney, and urinary bladder. Specimens of blood and saliva, collected about 1 hour before death, also produced paralysis and death in rabbits.

Identification of B virus recovered from monkey tissues was made by neutralization with B virus antiserum prepared in rabbits.

Summary

The data presented show that of five species of animals exposed to aerosols of monkey B virus, rabbits, monkeys, and guinea pigs are susceptible, rabbits being the most susceptible.

It appears that the immunity of monkeys may be counteracted by monkey B virus if the dose administered is sufficiently large. Stimulation in antibody production seems to occur when monkeys inhale sublethal doses of B virus. B virus was isolated from saliva and blood before the death of an infected monkey and was isolated from ten tissues after death.

ENTERIC BACTERIOLOGICAL STUDIES IN A LARGE COLONY OF PRIMATES*

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Diarrheal diseases consistently have been the major disease problem at the monkey-conditioning farm operated by the National Foundation for Infantile Paralysis at Okatie Farms, S.C. In 1953 A. V. Hardy, of the Commission on Enteric Infections of the Armed Forces Epidemiological Board, was called in for consultation to determine what could be done to reduce the high mortality experienced in the monkey colony at Okatie Farms. A preliminary report[1] of his findings was presented at the Animal Care Panel in 1954 in Chicago, Ill. His studies indicated that *Shigella* and *Salmonella* were widely present in these primates and that the sulfonamide drug therapy used at that time was not proving effective in reducing enteric infections or mortality. On the basis of these early findings and in order to determine more fully the nature and extent of enteric infections as they are related to diarrheal disease and mortality, intensive laboratory studies were initiated.

Okatie Farms was established to receive shipments of rhesus (*Macaca mulatta*) and cynomolgus (*Macaca cynomolgus*) monkeys from India and the Philippines, respectively, and to hold them for a period of conditioning before shipment to laboratories throughout the United States. There has been in recent years an urgent need for large numbers of monkeys for use in tissue culture procedures, for diagnostic studies, and for research in virology, particularly in poliomyelitis. During 1955 more than 60,000 monkeys were received at Okatie Farms.

The physical facilities at the farm originally were divided into four areas: (1) well area; (2) ready-for-shipment area; (3) hospital; and (4) recuperation area. Each shipment included about 1500 to 1800 animals. Upon arrival, the 80 to 100 animals contained in 6 to 8 small shipping crates were transferred to a large group cage in the well area to be held for 1 to 4 weeks, unless illness ensued. These "gang cages" were checked twice daily, and all apparently ill animals were removed to the hospital for specific therapy. Following clinical recovery from the illness, the animals were transferred to the recuperation area and later to the well area. Clinically healthy animals were placed in the ready-for-shipment area to await distribution. The maintenance in large gang cages and the repeated transfers provided ample opportunity for the spread of enteric infections. Later, the above plan was modified by instituting intramuscular injection of all animals with broad-spectrum antibiotics on their arrival. This was done to ensure mass prophylactic therapy on an individual basis with a minimum handling of animals. Also, in so far as practicable, animals received in one shipment were kept together at least until most of the animals were distributed. Rhesus and cynomolgus monkeys were always caged separately and in different areas if possible.

* The work described in this paper was supported in part by research grants from the National Foundation for Infantile Paralysis, New York, N.Y., and from the Department of Defense acting for the Commission on Enteric Infections, Armed Forces Epidemiological Board, Washington, D.C.

Bacteriological Procedures

The rectal swab technique described by Hardy *et al.*[2] for the collection of specimens and the direct inoculation of agar Petri plates and enrichment broth was used. The swabs were streaked to *Salmonella-Shigella* (SS) agar and then placed in tetrathionate broth with brilliant green added. After overnight incubation, a portion of the broth was streaked to a brilliant-green agar (BG) Petri plate. In some cases the original rectal swab was inoculated onto eosin-methylene-blue (EMB) plates for the isolation of *Escherichia coli*. To inhibit the growth of *Proteus*, it was found advantageous to add sulfathiazole to the tetrathionate enrichment broth and to the BG agar to a final concentration of approximately 0.125 mg. per cent.[3] This drug has relatively no effect on the growth of *Salmonella*, but inhibits materially the troublesome overgrowth with *Proteus*.

TABLE 1

DISTRIBUTION OF ENTERIC INFECTION BY "CONDITIONING STATUS" OF MONKEY

Conditioning area	No. examined	Positive					
		Total		*Shigella*		*Salmonella*	
		No.	Per cent	No.	Per cent	No.	Per cent
Receiving	126	11	8.7	3	2.4	8	6.35
Well	558	338	60.6	234	41.9	179	32.1
Hospital	1202	424*	35.3	180	15.0	258	21.5
Recuperation	196	127	64.8	116	59.2	19	9.7
Shipping	92	28	30.4	24	26.1	7	7.6
Total	2174	928	42.7	557	25.6	472	21.7

Monkeys with multiple infections observed in all areas except Receiving.
* Twenty-three monkeys negative for *Salmonella* and *Shigella* yielded *E. coli* 0-111.

Suspicious colonies were picked to Kligler's iron agar with 1 per cent sucrose added. Standard biochemical and serologic tests were employed to screen organisms and to identify the enteric pathogens.[2]

Findings

To determine the general distribution of enteric infections, rectal cultures were taken from animals in the several areas of the colony. The results are given in TABLE 1. *Shigella* and *Salmonella* infections were present in all 4 areas. There were many instances of multiple infections in the same animal. The over-all infection rates were 25.6 per cent for *Shigella* and 21.7 per cent for *Salmonella*. The lowest rates were found among the animals newly received on the farm. Thereafter the *Shigella* infections commonly spread rapidly, resulting in a five- to fifteenfold increase in the rate of infection among animals in the so-called well area. The proportion of animals found positive for *Shigella* was somewhat less in the hospitalized group, presumably due to the use of drug therapy during hospitalization. Soon after discharge to the recuperation area, the over-all infection rate increased markedly to a high of almost 65 per cent.

It was very apparent that monkeys often are asymptomatic carriers of *Shigella* and *Salmonella*, as indicated by the findings on 92 apparently healthy animals examined when ready for shipment: 28 (30.4 per cent) were positive for *Shigella* or *Salmonella*.

The isolation of *E. coli* 0-111, a coliform organism believed to have an etiological role in human infant diarrhea, was of interest. Of 75 hospitalized animals free of symptoms of dysentery and from which no *Salmonella* or *Shigella* were isolated, 23 were positive for *E. coli* 0-111. The significance of this observation is not known. Later studies indicated that this serotype, as well as *E. coli* 0-55, could be readily isolated from apparently healthy monkeys.

Inasmuch as there was a continuing influx of shipments of monkeys from the Orient, it appeared desirable to determine, on a planned basis, the amount of infection being introduced with each shipment. During 1955 rectal swab cultures were taken from a sample of each newly arrived shipment and examined

TABLE 2

ENTERIC BACTERIOLOGICAL FINDINGS ON MONKEYS NEWLY RECEIVED AT OKATIE FARMS DURING 1955

Season	Rhesus*			Cynomolgus†		
	Animals examined	Positive		Animals examined	Positive	
		Shigella	*Salmonella*		*Shigella*	*Salmonella*
Winter	572	88 (15.4)‡	9 (1.6)	276	18 (6.5)	36 (13.0)
Spring	1143	146 (12.8)	52 (4.5)	293	13 (4.4)	47 (16.0)
Summer	1042	395 (37.9)	216 (20.7)	450	6 (1.3)	92 (20.6)
Fall	436	58 (13.3)	62 (18.1)	200	15 (7.5)	13 (6.5)
Total	3193	687 (21.5)	356 (11.1)	1219	52 (4.3)	189 (15.5)

* A total of 36,248 rhesus monkeys (8.8 per cent sample size).
† A total of 24,261 cynomolgus monkeys (5.0 per cent sample size).
‡ Per cent expressed in bracketed figures.

for *Salmonella* and *Shigella*. The results of this study are presented in TABLE 2. During the period of observation, 36,248 rhesus and 24,261 cynomolgus monkeys were received at the farm and 8.8 and 5.0 per cent, respectively, were examined culturally. The *Shigella* infection rates for the rhesus monkeys varied from 12.8 to 15.4 per cent for all seasons except summer, when the rate increased markedly to 37.9 per cent. *Salmonella* infections among the rhesus monkeys were more prevalent in the summer and fall. By comparison, in the cynomolgus monkeys, the over-all infection rate with *Shigella* was relatively low (1.3 to 7.5 per cent), with comparatively little variation between seasons; however, the rates for *Salmonella* infections were higher than for *Shigella*. The *Shigella* infection rates for the rhesus were 5 times as high as for the cynomolgus. This study established the fact that infection with enteric bacteria that certainly are harmful to man and that may be pathogenic for primates were common in these new arrivals.

Mortality records on the animals received at the farm in 1955 were compared with infection rates (TABLE 3). There was comparatively little seasonal varia-

tion in mortality among the rhesus except during the fall, when the mortality dropped from the usual 8 per cent to 3 per cent. In contrast, there was a marked increase in mortality among the cynomolgus monkeys during the fall and winter as compared to the other seasons of the year. This mortality was associated with pneumonia, although diarrheal disease was in evidence throughout the year. Comparing infection rates and mortality, the findings did not suggest that the latter varied with the former. However, it was noted that in animals with acute diarrheal disease, the enteric cultures commonly revealed large numbers of suspect organisms, seemingly almost pure cultures of the pathogen. This was in contrast to findings on the apparently healthy animals, where the usual findings were scattered suspect colonies on the inoculated plates. Evidently, other factors in addition to infection with these enteric pathogenic bacteria contributed to mortality. Presumably these included

Table 3
Comparison of Mortality* and Infection Rates in Monkeys Received at Okatie Farms

Season	Rhesus: Number of animals: Received	Rhesus: Number of animals: Deaths*	Rhesus: Per cent mortality	Rhesus: Per cent infections†: *Shigella*	Rhesus: Per cent infections†: *Salmonella*	Cynomolgus: Number of animals: Received	Cynomolgus: Number of animals: Deaths*	Cynomolgus: Per cent mortality	Cynomolgus: Per cent infections†: *Shigella*	Cynomolgus: Per cent infections†: *Salmonella*
Winter	6881	594	8.63	15.4	1.6	5607	868	15.5	6.5	13.0
Spring	12141	884	7.28	12.8	4.5	5879	352	5.99	4.4	16.0
Summer	12557	1136	9.05	37.9	20.7	8932	666	7.46	1.3	20.6
Fall	4669	146	3.13	13.3	18.1	3843	991	25.8	7.5	6.5
Total	36248	2760	7.61	21.5	11.1	24281	2877	11.8	4.3	15.5

* Accumulated number of deaths observed during first 8 days.
† Per cent infections of animals examined. Data taken from TABLE 2.

nutrition, virus and parasitic infections, and the general physical and also the emotional health status of the animal.

Illness and death were common in animals in some shipments and almost nonexistent in others. One shipment of 1,936 cynomolgus monkeys received during a cold spell in November 1955 suffered a loss of 39.4 per cent within the first 8 days on the farm. Most of the deaths were associated with pneumonia; there was relatively little evidence of enteric disorder in this shipment.

The distribution of enteric pathogens isolated from monkeys examined on arrival at Okatie Farms is presented in TABLE 4. The predominating type of *Shigella* was *Sh. flexneri 2*, followed by *Sh. flexneri 4*. Other types found included *Sh. dysenteriae 2* (Schmitz), *Sh. sonnei*, and *Sh. flexneri 6*. Thirteen different types of *Salmonella* were found. The predominating types were *S. anatum*, *S. stanley*, and *S. typhimurium*. The particular types of *Shigella* and *Salmonella* varied from shipment to shipment and from month to month. Paracolon strains and pathogenic serotypes of *E. coli* were also isolated.

Limited data were obtained on the natural spread of enteric infections among untreated groups of animals. Representative animals of 3 separate shipments (TABLE 5) were tagged at the time the initial enteric cultures were obtained. Repeat cultures were taken at 5- to 7-day intervals during their stay on the

TABLE 4

DISTRIBUTION OF ENTERIC PATHOGENS ISOLATED FROM 3250 MONKEYS* CULTURED ON ARRIVAL AT OKATIE FARMS

Genus	Type	Number
Shigella	*dysenteriae* 2	86
	flexneri 2	558
	flexneri 4	97
	flexneri 6	32
	sonnei	38
Total *Shigella*		811
Salmonella	*anatum*	126
	stanley	43
	typhimurium	42
	newport	23
	potsdam	9
	montevideo	4
	paratyphi B (var. *Java*)	3
	(Other)†	6
Total *Salmonella*		256
Grand total		1067

* 2406 rhesus and 844 cynomolgus.
† *S. infantis*, *S. javiana*, *S. tennessee*, *S. darby*, *S. give* and *S. oranienburg*.

TABLE 5

NATURAL SPREAD OF ENTERIC INFECTIONS AMONG UNTREATED TAGGED ANIMALS

Group	No. days caged together	No. tagged animals examined*	Per cent positive†	
			Shigella	*Salmonella*
R 26 (rhesus)	1	100	20	39
	5	98	34	21
	13	75	53	4
	22	70	60	1
	28	59	44	0
	34	46	24	0
R 60 (rhesus)	1	104	2	10
	10	102	15	3
	15	93	25	17
	23	85	31	5
C 29 (cynomolgus)	1	100	0	8
	5	115	17	55
	13	50	28	6

* Some animals were lost to study through loss of tags or death or were shipped out.
† Prevalence of *Shigella*- and *Salmonella*-infected animals based on a single culture taken on the day indicated.

farm. In shipment R26 there was a build-up in the prevalence of shigellosis during the first 3 weeks, and thereafter a decline. However, at the end of the period of observation, a higher percentage of animals was infected with *Shigella* than at the time of their arrival on the farm. The findings for *Salmonella*, however, were different. Thirty-nine per cent were infected at the time of arrival, but thereafter there was a relatively rapid decline, and the last positive cultures for *Salmonella* in these animals were found on the twenty-second day after arrival. These examinations were repeated with 2 other shipments (R60 and C29); unfortunately, the times of observation were limited to 23 and 13

TABLE 6
BACTERIOLOGICAL RESPONSE OF *SHIGELLA* INFECTIONS TO DRUG THERAPY IN MONKEYS*

Series	Therapy (total dose)	Cultures taken days after beginning therapy	Treated		Untreated	
			No.	Per cent positive	No.	Per cent positive
A	Terramycin, oral,† 400 mg., 8 days	1 8	18	72 31	8	50 25
B	Terramycin, oral, 900 mg., 9 days	1 3 5 9	9	100 22 0 0	8	100 62 75 12
C	Terramycin,‡ I.M., 75 mg., 1 injection	1 2 7 8	36	64 0 4 4	47	64 71 14 25
D	Chloramphenicol,§ I.M., 100 mg., 1 injection	1 2 6	15	100 67 0	10	80 100 50
E	Entromycin, oral, 40 gm., 10 days	1 2 7 10	26	81 16 25 0	9	56 25 22 14

* Average weight per monkey approximately 2.0 to 3.0 kg.
† Oral Terramycin (Pfizer) and Entromycin (Pitman-Moore) given in drinking water or in wet feed.
‡ Pfizer.
§ Parke-Davis.

days, respectively. In both, there was the progressive increase in the prevalence of shigellosis, while the prevalence of salmonellosis varied. These animals were housed in the large gang cages, and there were obvious opportunities for spread of infection. The course of these infections in groups of primates in captivity closely corresponds with that observed in human populations in institutions. Watt[4] has reported that under these circumstances *Shigella* infections tend to spread readily, to reach a peak, and thereafter to decline slowly, whereas *Salmonella* infections do not spread readily and generally disappear spontaneously.

The response to therapy was evaluated bacteriologically; data are presented in TABLE 6. Medication was given by the oral route and by injection. In

series A and B, Terramycin at 150-mg./kg. and 300-mg./kg. levels was tried. Apparently, the higher level served to rid or at least suppress the *Shigella* infections in the treated animals. In series E, entromycin given orally was much less effective in reducing the *Shigella* infection; however, it appeared to reduce the severity of diarrhea. Our over-all experience with drug therapy established that attempts to provide medication to seriously ill animals in food or water was not too effective. Therefore, the value of intramuscular injection of the broad-spectrum antibiotics was examined. In series C and D, terramycin and chloramphenicol were tried. Both appeared to be effective in ridding (or suppressing) the animals of the infection. Later experience with the use of these drugs suggests that animals may stop shedding *Shigella* following drug therapy, but resume shedding the same type several days after the therapy has been discontinued. None of the broad-spectrum drugs used proved to have any significant effect on *Salmonella* infections. Generally speaking, broad-spectrum antibiotics when given intramuscularly were effective in reducing mortality in monkeys during treatment and for a short period thereafter. Mortality increased after a week or 10 days following the last injection.

In conclusion, it must be stated that the control measures instituted on the basis of the information obtained have not proved effective in the control of enteric infections at Okatie Farms. This was true even though those responsible for the care of animals applied vigorous methods designed to limit the spread of enteric infections. The entire area was cleaned carefully each day; the methods of feeding and provision of drinking water were designed to limit in so far as possible contamination with feces; handling of animals was kept to a minimum; prophylactic medication was provided by intramuscular injection of broad-spectrum antibiotics when tuberculin-testing; sick animals were housed in a sheltered enclosure; and there was a general effort to promote the health and well-being of captive monkeys to ensure their usefulness for research. Despite this care, enteric infections spread actively among the primates, as might have been expected with the intimacy of contacts that was an inevitable part of housing in large group cages. We were forced to acknowledge that, under these conditions, the spread of enteric infections among primates could not be controlled effectively.

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ENTERIC VIRUSES OF MONKEYS*

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Extensive use of the monkey in the production and testing of poliomyelitis vaccine and in other experimental studies in recent years has created an urgent need for a better understanding of the causes of disease in this animal. Studies on the viral flora of monkeys have shown that they serve as hosts for a large number of such agents. While viruses have been isolated from many of the organs of the monkey, the intestinal tract serves as a particularly rich source of these organisms. Although many of these simian intestinal agents resemble in some of their properties either the entero- or adenoviruses of humans, their role as causative agents in diseases of the monkey remains unknown. Considerable interest is being shown in them, however, and several studies are now in progress that may shed some light on this problem.

It is well known that both rhesus and cynomolgus monkeys held in captivity are prone to diarrheal disease, which at times may be associated with a high case fatality rate. The etiology of this disease (or diseases) remains obscure; in all probability it involves more than one cause. Members of the *Shigella* and *Salmonella* genera are frequently found in association with diarrheal disease but, as described by Schneider elsewhere in this monograph, there is no clear-cut evidence that these organisms are the responsible etiological agents.

The multiplicity of viral agents inhabiting the simian intestinal tract suggested that members of this group might be playing a significant role in the pathogenesis of the disease observed. With this in mind Hoffert *et al.*[1] at the University of Pittsburgh, Pittsburgh, Pa., studied the prevalence of viral agents in the intestinal tracts of normal monkeys and of monkeys with diarrheal disease. They found no correlation between the occurrence of diarrheal disease and the presence of a specific virus, either alone or in association with *Shigella* organisms. Viral agents were as prevalent in normal monkeys as in monkeys with diarrheal disease. In both groups isolation rates of approximately 90 per cent were observed. By antigenic analysis these investigators identified 13 prototype viruses among the 169 strains isolated, but were unable to demonstrate any *specific* serologic relationship between these simian enteric viruses and human enteroviruses. Seven of the prototype viruses, however, contained the human adenovirus *group* complement-fixing antigen.

Other workers also have demonstrated the presence of simian enteric viruses in both diseased and apparently healthy monkeys. Thus, Hull and his co-workers[2] have isolated viral agents from rectal swabs taken from monkeys with diarrheal disease. Riordan, as cited by Hsiung and Melnick,[3] isolated 25 viral strains from the stools of 21 apparently healthy monkeys. Rowe *et al.*[4] isolated several kinds of adenoviruslike viruses from simian sources, including the in-

* The work reported in this article was initiated under the sponsorship of the Commission on Enteric Infections of the Armed Forces Epidemiological Board, Washington, D.C. and by grants from the Office of the Surgeon General, Department of the Army, Washington, D.C., The National Foundation, New York, N.Y. and the National Institute of Allergy and Infectious Diseases, Public Health Service, Bethesda, Md.

testinal tract. Albano,[5] in a study of 29 normal rhesus and cynomolgus monkeys, recovered 27 viral agents from their feces.

In addition to these studies of the intestinal viral flora, work done in the laboratories of R. N. Hull, of H. Malherbe, and of N. J. Schneider has resulted in the isolation of a large number of viral agents, some identical with recognized simian enteric viruses from tissues other than those of the intestinal tract. Hull and his co-workers[2,6,7] have isolated the large majority of their viruses from uninoculated monkey kidney cell cultures with a few additional isolates from central nervous system tissue. Malherbe and Harwin[8] isolated 7 viral agents from uninoculated vervet monkey kidney cell cultures. N. J. Schneider, in his studies of moribund monkeys, has isolated viruses in significant numbers from heart, spleen, lung, pancreas, kidney, and plasma (personal communication, 1959).

TABLE 1

RELATIONSHIP OF PROTOTYPE VIRUSES TO OTHER SIMIAN VIRUSES

Prototype virus	Simian virus group (Hull)	Simian adenovirus group (Rowe)	ECHO virus group (Melnick)
P1	S.V.$_{2}$	Not tested	
P2	S.V.$_{19}$*	Not tested	
P3	S.V.$_{6}$	Not tested	Rh 7998
P4	S.V. $_{0}$*	?†	
P5	S.V.$_{30}$*	No number assigned*	
P6	S.V.$_{31}$*	PD 2054	
P7	S.V.$_{32}$*	M-3	
P8	S.V.$_{23}$	M-2	Rh 7853, Rh 7854, Rh 7855
P9	S.V.$_{36}$*	?	
P10	S.V.$_{33}$*	?	
P11	S.V.$_{18}$	Not tested	Cy 7849
P12	?	Not tested	
P13	?	Not tested	Cy 8006

* New type, provisional designation.
† Under study.

The relationship between the prototype viruses of W. R. Hoffert *et al.* and the viruses isolated by R. N. Hull, W. P. Rowe, and J. L. Melnick is shown in TABLE 1. Schneider has also found a number of his viral strains to be identical with certain recognized prototypes, that is, P1, P2, and P3 (personal communication, 1959). It is apparent that at least some of the simian prototype strains are widespread in their occurrence.

The presence of "enteric" viruses among those isolated from nonintestinal sources by Hull and by Schneider suggests that some of these isolations may have resulted from agonal viremia in a moribund monkey or contamination from the intestinal tract or coat of the animal. Our associate, M. B. Dobkin, has determined the isolation rate of viral agents from various tissues of a relatively small group of apparently healthy monkeys. Extreme care was taken to prevent contamination. The results are given in TABLE 2. Of the individual tissues examined, 3.8 per cent of the spleen specimens, 3.6 per cent of the kidney specimens, and 27 per cent of the intestinal wall specimens were positive. All other tissues were negative, while 58 per cent of the intestinal content speci-

mens examined yielded viral agents. The low isolation rates from visceral organs, as compared to those reported by Schneider in his studies of moribund monkeys may well reflect the state of well-being of our monkeys, who had benefited from a long conditioning period.

In the hope that new information might be obtained on the status of the simian enteric viruses as causal agents of disease, we are participating in two longitudinal studies of monkeys held in captivity.

The first, which is being carried out in conjunction with Merck Sharp & Dohme, West Point, Pa., and the School of Veterinary Medicine of the University of Pennsylvania, Philadelphia, Pa., involves a group of 40 newly imported rhesus monkeys. These monkeys were quartered in two gang cages affording access to a larger group of approximately 160 monkeys through the wire mesh of the cage. The monkeys were observed for a period of 12 weeks; during this period weekly rectal swabs were taken in an attempt to isolate viral agents and

Table 2
Summary of Viral Isolations Made from Organs of Rhesus Monkeys

Specimen tested	No.	Positive (no.)	Positive (%)
Heart	48	0	0.0
Lung	48	0	0.0
Liver	53	0	0.0
Spleen	53	2	3.8
Kidney	27	1	3.6
Serum	25	0	0.0
Lymph node	2	0	0.0
Tonsil	4	0	0.0
Intestinal wall	47	13	27.0
Total	308	16	5.3
Intestinal contents	53	31	58.5

members of the *Salmonella* and *Shigella* group. Serum specimens for antibody studies were obtained at regular intervals. During examination of dead or moribund animals, tissue specimens were taken from selected organs in an attempt to isolate viruses. To date, we have processed 353 rectal swabs by the inoculation of monkey kidney cell cultures and have obtained a virus isolation rate of 88 per cent. We are now in the process of identifying these agents as the first step in determining their role in the pathogenesis of the disease observed.

The second study in which we are participating is a cooperative one sponsored by the National Advisory Council on Primates, Bethesda, Md. Its purpose is to study the causes of disease in a group of 600 monkeys newly imported from India and observed for a period of 8 weeks under different conditions of housing. No significant data are thus far available from this study.

Hoffert and his associates[1] have described some of the properties of the 13 prototype viruses identified by them. These have been divided into two groups on the basis of their characteristic cytopathogenic effects (CPE) on monkey kidney cell cultures. Types P4, P5, P6, P7, P8, P9, and P10 produce

a delayed CPE resembling that caused by human adenoviruses. These 7 agents, which possess the adenovirus group antigen, comprise CPE group 1. On the other hand, P1, P2, P3, P11, P12, and P13 resemble human enteroviruses in the type of CPE that they produce. Of these six prototypes comprising CPE group 2, P2 and P13 are pathogenic for suckling mice, but as yet no antigenic relationship to recognized members of the Coxsackie group has been demonstrated.

FIGURE 1. Plaques typical of those formed on rhesus monkey cell monolayers by CPE group 1 prototype viruses.

More recently, further studies of the physical and biological properties of these agents have been carried out in our laboratory. A brief summary of the results to date follows.

A study of the plaque characteristics of the prototype viruses has shown that they may be classified further on the basis of their plaque morphology.

The CPE group 1 viruses produced clear round plaques that reached a size of 2 to 4 mm. in 14 days under a 1 per cent agar overlay medium on monkey kidney monolayers (FIGURE 1). No significant differences were noted in the plaque characteristics of individual viruses, with the exception of the time of appearance, which ranged from 6 to 11 days. Because of their delayed appearance, plaques of these viruses were difficult to obtain.

CPE group 2 viruses, on the other hand, were less alike in the type of plaque they produced. These agents fell into 2 broad groups: one producing plaques

similar in their sharpness to those of polio and Coxsackie virus, the other producing plaques similar to those of the ECHO viruses. The plaques formed by the agents in the first group (P1, P3, and P11) were large and round, with clearly defined edges. The presence of a few islets of viable cells within the periphery of the plaque was quite distinctive. These plaques appeared on the second or third day after inoculation. The second group of viruses (P2, P12, and P13) produced a less distinct plaque characterized by the presence of many viable cells within a border that was not too clearly defined, thus giving the plaques a more diffuse appearance (FIGURE 2). These plaques appeared on the fourth or fifth day after inoculation.

These two distinctive patterns of plaque formation among CPE group 2 viruses may be correlated with other properties such as growth characteristics under agar overlays with high and low bicarbonate concentrations, thermal stability, and growth on *Erythrocebus patas* monkey kidney cells.

When P1, P3, and P11 were plated on monkey kidney cell monolayers with agar overlays containing 0.11 and 0.45 per cent sodium bicarbonate, they produced similar numbers of plaques in both instances. P2, P12, and P13, on the other hand, produced approximately 10 times as many plaques under the high bicarbonate overlays as under the low (TABLE 3). When the *p*H and bicarbonate concentration were varied, the effects observed indicated that the phenomenon was a reflection of the optimal *p*H requirements for plaque formation.

The thermal inactivation curves for the CPE group 2 viruses showed that they could be divided into 2 groups on the basis of their thermal stability at 45° C. P1, P3, and P11 were rather rapidly inactivated, whereas P2, P12, and P13 were relatively stable at this temperature, thus resembling CPE group 1 viruses, which were uniformly resistant to inactivation at 45° C. (FIGURE 3).

As previously shown by Hsiung and Melnick,[3] the simian enteric viruses may be grouped according to their ability to produce plaques on rhesus and *patas* monkey kidney cell monolayers. These investigators found that the CPE group 1 viruses were uniform in the production of delayed plaques on both rhesus and *patas* monolayers. Of CPE group 1 strains, P1, P3, and P11 as well were shown to produce plaques on the two types of cells, while P2 alone was not capable of producing plaques on *patas* cells. We have confirmed these results and in addition have found that P13 does not produce plaques on *patas* cells, while P12 forms delayed, small, diffuse plaques that appear on the eighth day after inoculation.

In summary, the CPE group 1 viruses are uniform in the characteristics studied. The CPE group 2 viruses, on the other hand, can be divided into two subgroups. One group (P1, P3, and P11), which produced sharp round plaques with islets of viable cells within their border on rhesus kidney cells, was uniform in plaque production under acid and alkaline agar overlays, was sensitive to heat inactivation, and formed plaques on rhesus and *patas* monkey kidney cell monolayers; another group (P2, P12, and P13), which produced diffuse plaques on rhesus kidney cells, had a reduced plaquing efficiency on acid media, was more resistant to heat inactivation and, with the exception of P12, did not form plaques on *patas* kidney cells.

Additional studies have been carried out by our associate, A. S. Abraham,

on the growth characteristics and hemagglutinating properties of these viruses. In general, data on the rates of adsorption and multiplication of the various prototype viruses showed that the CPE group 1 agents resembled the human adenovirus group, while members of the CPE group 2 resembled human enteroviruses. Among the CPE group 1 prototype strains, P6 agglutinated human type O blood cells, and monkey, chick, guinea pig, rabbit, and sheep red blood

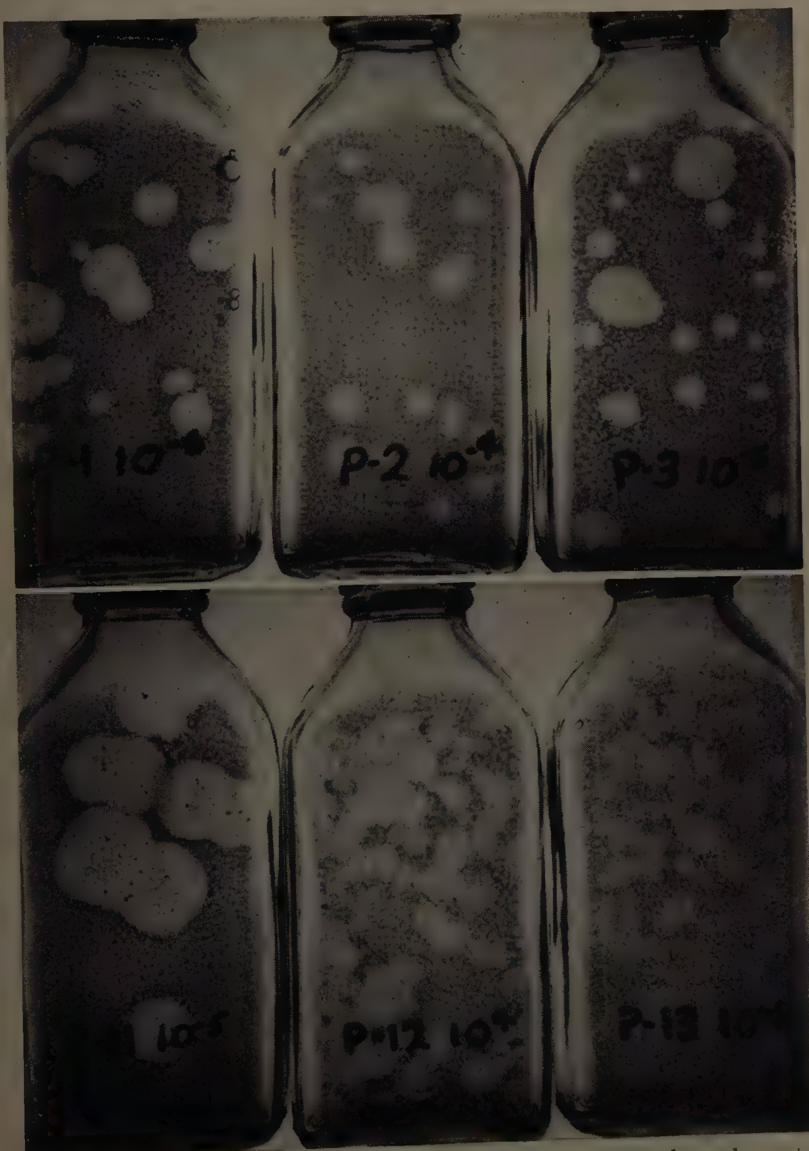

FIGURE 2. *Top*, plaques formed on rhesus monkey kidney cell monolayers by prototype viruses P1, P2, and P3, 7 days after inoculation. *Bottom*, plaques formed on rhesus monkey kidney cell monolayers by prototype viruses P11, P12, and P13, 7 days after inoculation.

cells to a moderate titer (1:64). P9 agglutinated simian red blood cells only to a similar titer. The other prototype strains yielded negative results. Among the CPE group 2 prototypes, P11, P12, and P13 agglutinated rabbit cells weakly, the maximal titer being 1:8. In general, these results are in accord with those reported by Hull *et al.*[2]

TABLE 3
EFFECT OF BICARBONATE CONCENTRATION ON FORMATION OF PLAQUES

Virus	Plaque-forming units determined at end of 7 days	
	0.11% bicarbonate*	0.45% bicarbonate*
P1	14.7×10^6	14.2×10^6
P2	$<1.0 \times 10^4$	12.5×10^4
P3	21.0×10^5	14.2×10^5
P11	19.3×10^5	18.8×10^5
P12	$<1.0 \times 10^4$	10.2×10^4
P13	$<1.0 \times 10^4$	2.7×10^4

* Percentage of sodium bicarbonate in the agar overlay medium.

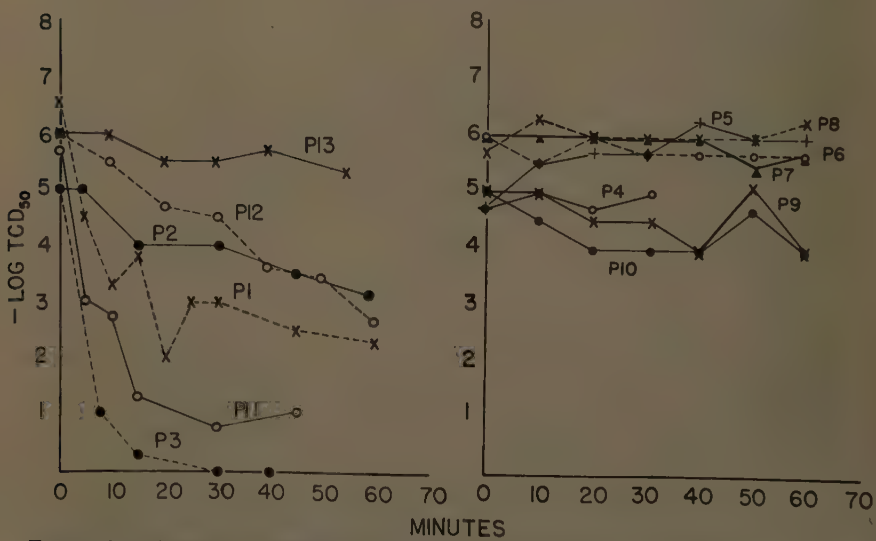

FIGURE 3. Thermal inactivation curves (45° C.) for CPE groups of prototype viruses. *Left*, CPE group 2; *right*, CPE group 1, TCD_{50}.

Discussion and Conclusions

We have briefly described some of the properties of a group of 13 prototype viruses isolated from the intestinal tracts of normal monkeys and monkeys with signs of diarrheal disease. At present little knowledge is available as to the relationship of these agents to disease in the monkey. These viruses have been isolated from the gastrointestinal discharges of both healthy and ill monkeys and from various organs of moribund animals. Hull *et al.*[7] have reported

that several of their enteric viruses cause death of the monkey when inoculated intracerebrally and that, of the several viral agents isolated from central nervous system tissue, one possessed the characteristics of an enteric virus. Two of the agents in the prototype group discussed in this paper produce Coxsackielike lesions in suckling mice, but no relationship has been shown between these agents and a disease in monkeys. To the best of our knowledge, no reports have been made of the experimental production of disease by the inoculation of simian enteric viruses with the exception of Hull's work, cited above.[7]

It has been pointed out that none of the prototype viruses discussed in this paper has shown a specific antigenic relationship to human entero- or adenoviruses. On the other hand, Hull *et al.*[2] have isolated from the intestinal tract of monkeys a virus (S.V.$_{12}$) that is antigenically related to Reovirus Type I (ECHO 10),[9] indicating that some of the simian enteric viruses may be of human origin.

Enteric viruses have been isolated from the gastrointestinal tracts of a high percentage of apparently healthy monkeys and as such may have established a commensal relationship with their hosts similar to that of the colon bacilli. It is the purpose of the studies in which we are currently engaged to determine whether these apparently harmless viruses can become pathogenic under certain conditions and, if so, what diseases they produce.

The simian enteric viruses are of concern also because of the confusing picture they may give in experimental studies involving the inoculation of monkeys with other viral agents. The possibility of the occurrence of enteric viruses in organs other than the gastrointestinal tract must be kept in mind. In feeding experiments involving monkeys the excreted virus must be identified so as to rule out contamination from the host viral flora.

Summary

Apparently healthy monkeys, as well as those suffering from diarrheal disease, show a high prevalence of enteric viruses in their gastrointestinal discharges. On occasion they have been isolated from visceral organs other than the intestinal tract. To date, none of these viral agents has been related to a specific simian disease. These simian enteric viruses are similar to human enteroviruses or human adenoviruses in some of their properties but, with the exception of Hull's S.V.$_{12}$, no specific antigenic relationships have been noted.

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A POX DISEASE OF MONKEYS

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The susceptibility of the monkey to experimental infection with pox viruses of the variola-vaccinia group has been known for many years,[1] and macaque species have been used for research on these agents. It is of particular interest that, although variola has been a disease of major importance in man since at least 251 A.D.,[2] natural occurrence of the disease in Old World monkeys has not been reported. In fact, the only report of the natural disease in monkeys was in 1922,[3] when it had occurred in New World monkeys (*Mycetes* and *Cebus*) in South America during an outbreak of smallpox in humans.

It is of further interest that no pox disease of monkeys of any type was reported again until this year, when von Magnus *et al.*[4] described the occurrence of a spontaneous disease in captive *Macacus cynomolgus* during the summer and fall of 1958. In February 1959 we[5,6] observed an outbreak of pox disease in a colony of monkeys in the United States and, although identity of the etiological agents of both outbreaks has not been established by simultaneous laboratory studies, characterization of the agents separately leaves little doubt that both outbreaks were due to the same virus. The present report details the salient features of the disease, its pathology, and the etiological agent.

Natural Hosts

In the original outbreak reported by von Magnus *et al.*[4] only cynomolgus monkeys were involved clinically, although *M. mulatta* also were present in the colony. The United States cases[5] were in both *M. philippinensis* (cynomolgus) and *M. mulatta* monkeys. Serologic studies in the latter experience have shown that a large number of rhesus monkeys apparently have had contact with the virus without showing clinical evidence.

Experimental Hosts

Rabbits. Scarification of the skin followed by application of virus results in the production of either pustular or confluent lesions, depending upon the concentration of virus, and a dilution of 1000 to 80,000 of egg-grown virus will produce pustules in rabbit skin by this procedure.[4]

Virus also is infective for rabbits when given by the intravenous, intradermal, and subcutaneous routes. Following intradermal injection, a primary pustular lesion frequently develops, followed by secondary pustules over various parts of the skin. Intravenous injection results in generalized disease, although the majority of adult animals infected by this route recover after

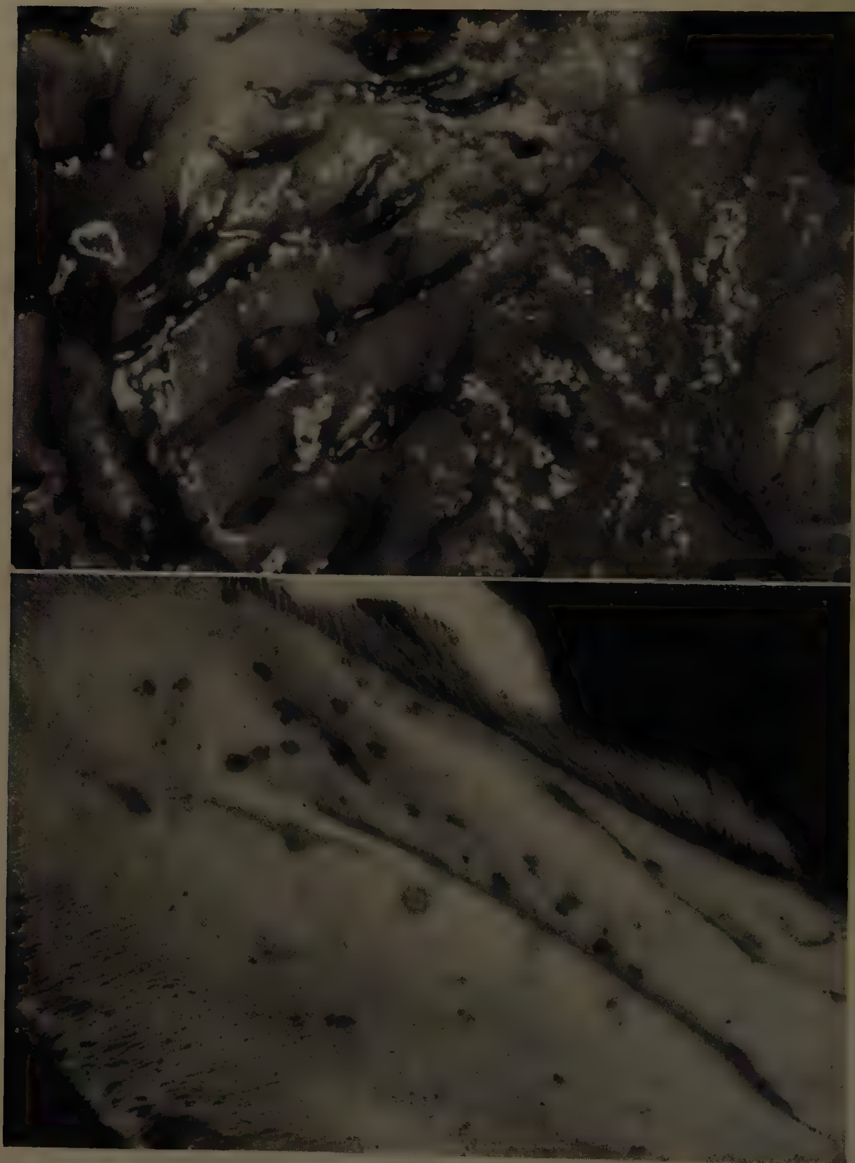

FIGURE 1. *Left*, inner surface of a rabbit ear following intravenous injection of monkey pox virus. *Right*, subcutaneous tissue of a rabbit injected intravenously with monkey pox virus, showing edema and hemorrhagic lymph tracts.

two or three weeks. However, animals in the acute phase that are examined post-mortem show pustules over most areas of the skin, subcutaneous edema with hemorrhagic, enlarged lymph tracts and, occasionally, subcutaneous circumscribed necrotic pustulelike lesions (FIGURE 1).

The virus of monkey pox can be passed in rabbits in serial passage by scarification. There is no apparent loss in virulence in subsequent passes.

Von Magnus *et al.*[4] were able to infect two-day-old rabbits. Administration of virus by intracutaneous or scarification routes resulted in death in five to seven days, grayish white spots were produced in the liver, and the kidneys had hemorrhagic foci. Lesions in these animals were indistinguishable from those produced by vaccinia virus. These workers also demonstrated susceptibility of the rabbit cornea to monkey pox virus.

Mice. Three-week-old mice are resistant to intraperitoneal injection of monkey pox virus, but intracerebral injection into three- to six-week-old mice

TABLE 1

INTRACEREBRAL PASSAGE OF MONKEY POX VIRUS IN MICE

Injected in 1959 (mo./day)	Strain	Age of mice (weeks)	Passage no.	Brains harvested* in 1959	No. dead/ no. injured	Recovery of virus in tissue culture
3/20	1744	3	1	3/25	1/10	Not done
3/25	1744	3	2	3/29	10/10	Not done
3/30	969	3	1	4/3	4/10	Not done
4/20	969	4	4	4/24	4/10	Not done
4/29	969	6	6	5/5	4/10	Positive
9/11	766	3	1	9/15	8/10	Not done
9/22	766	3	4	9/25	5/10	Positive
10/5	766	6	5	10/12	10/10	Not done

* Harvested only from surviving mice. Usually animals were visibly sick at time of harvest.

causes fatal infection. The virus has been serially passed at least six times by this route without change in virulence. TABLE 1 outlines several series of mouse passages.

Suckling mice are susceptible to intranasal inoculation,[4] death occurring five to fourteen days after administration of the virus.

Guinea pigs. Intravenous, intraperitoneal, subcutaneous, or intradermal injection of the monkey pox virus does not produce symptoms or lesions of disease. Injection of the foot pad, however, results in swelling in seven to ten days and development of a granulomatous lesion that remains confined to the injected area.

Monkeys. Both cynomolgus and rhesus monkeys are susceptible to the virus administered by the intradermal, subcutaneous, and intravenous routes. Intravenous injection results in generalized eruptions, and virus has been recovered from these lesions. Subcutaneous injection has produced a granuloma similar to that produced in the foot pads of guinea pigs. Intracutaneous injection causes local lesions without spread to other parts of the body.

The Clinical Disease

There have been two basic types of disease in spontaneous outbreaks of monkey pox observed to date, but it should be emphasized that complete detailed clinical data on the natural disease has not been accumulated.

The first clinical type consists of an acute disease, characterized by marked facial edema that extends to the cervical region. Severe difficulty in respiration is experienced and the animal dies, apparently of asphyxiation. At the same time, papular eruptions are present over various parts of the body, ulcerative lesions of the oral mucus membrane have been seen, and a generalized lymphadenopathy is present. This particular form of the disease has been seen only in cynomolgus monkeys.

TABLE 2

ISOLATION OF MONKEY POX VIRUS, STRAIN 1744

Naturally infected *M. philippinensis*

- Vesicular lesions in rabbit skins
- Primary rabbit kidney cells: inclusion bodies, degeneration
  - Primary rabbit kidney cells: inclusion bodies, degeneration
    - I.V. in rabbits: generalized pox
    - Monkey kidney cells: inclusion bodies, degeneration
    - Monkey inoculation: scarified skin; local vesicular lesion. I.V., generalized pox
    - Chorioallantoic membrane: discrete pocks. Hemagglutinin produced and complement-fixing antigen produced
      - Chorioallantoic membrane: titer 10^{-4}
    - Mouse injection: I.C., positive; I.P., negative

The second, more common, form of the disease shows only a cutaneous eruption and, except for apparent irritation from some of the local lesions, there are no other symptoms. Initially, there is usually a single crop of discrete papules ranging from 1 to 4 mm. in diameter, and the lesions then become pustular, containing a thick gray purulent material that can be expressed on pressure. Reddish-brown crustations form over the lesions and these drop off in 7 to 10 days, leaving small scars. A tendency of the lesions to be hemorrhagic was noted in fatal cases and in young animals. Although all parts of the body surface may be involved, the most common sites are buttocks, hands, feet, face, and hind limbs.

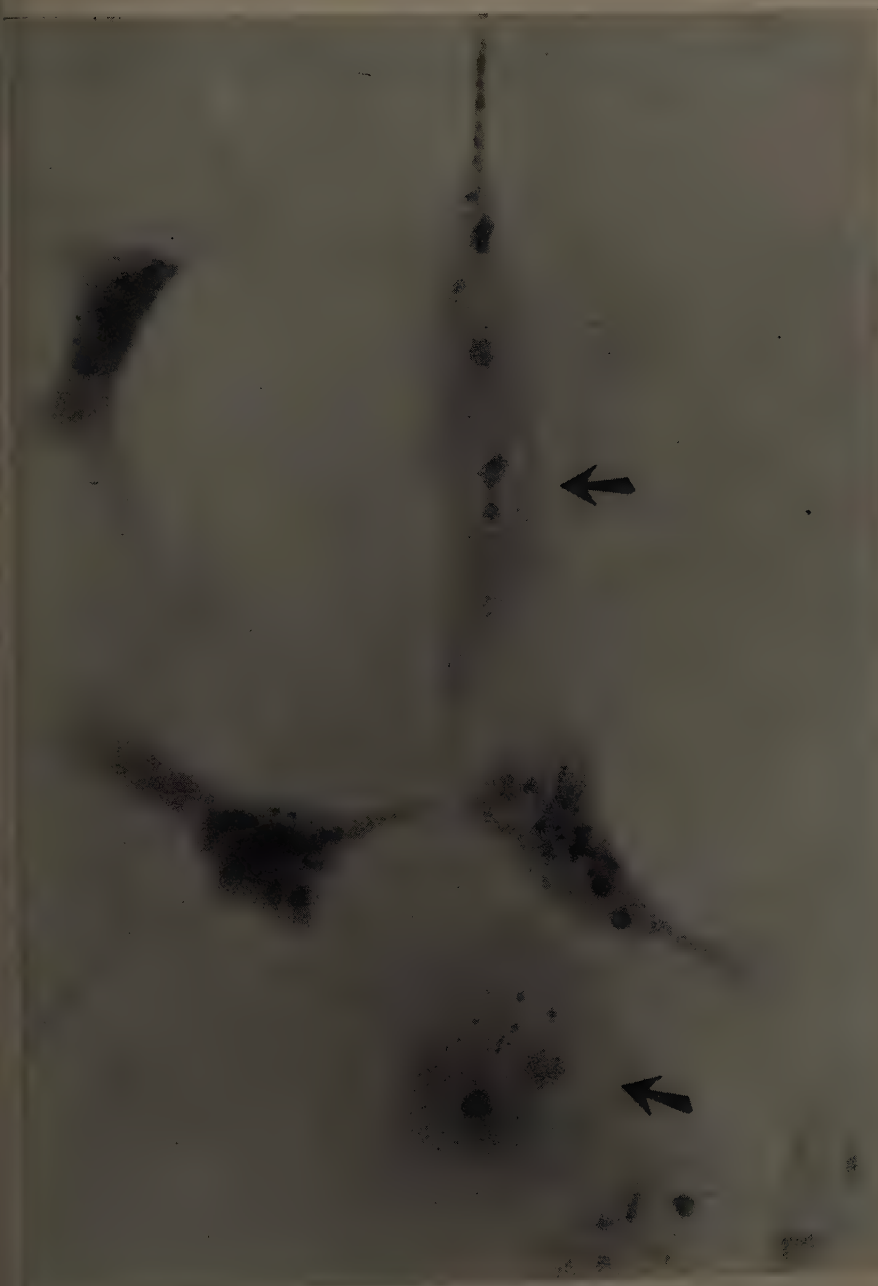

FIGURE 2. Monolayer cell culture of rabbit kidney epithelium, showing intracytoplasmic eosinophilic inclusion bodies as a result of infection with monkey pox virus, strain 1744.

Histopathology

The microscopic appearance of the skin is characterized by focal proliferation of the epidermis, followed by necrosis. Intracellular edema produces a great increase in the size both of cell bodies and of nuclei, but rarely proceeds to reticular degeneration before the onset of necrosis. Large vesicles therefore are occasionally seen, but are not common.

Invasion of edematous squamous cells by neutrophils results in the formation of spongiform pustules, and frequently the entire epidermis in a lesion

TABLE 3

ACTIVE PROTECTION OF MICE AGAINST MONKEY POX VIRUS

Immunization virus	Route of immunization	Results*
Vaccinia	I.C.	1/11
Monkey pox	I.P.	2/10
Control (saline)	I.C.	7/10

* Number dead/number inoculated. Challenge virus = 0.03 ml. monkey pox virus (undilute tissue-culture fluid) intracerebrally, 23 days after immunization.

FIGURE 3. Electron photomicrograph of monkey pox virus. ×20,000.

becomes necrotic. Subsequent liquefaction of the debris by polymorphonuclear leukocytes results in the formation of large pustules.

Intracytoplasmic inclusion bodies are numerous in epidermal cells along the sides of a lesion. They are 3 to 7 μ in diameter, round, eosinophilic, frequently multiple, and usually surrounded by a clear halo. Eosinophilic intranuclear inclusion bodies may also be seen, but never simultaneously in a cell with intracytoplasmic inclusion bodies.

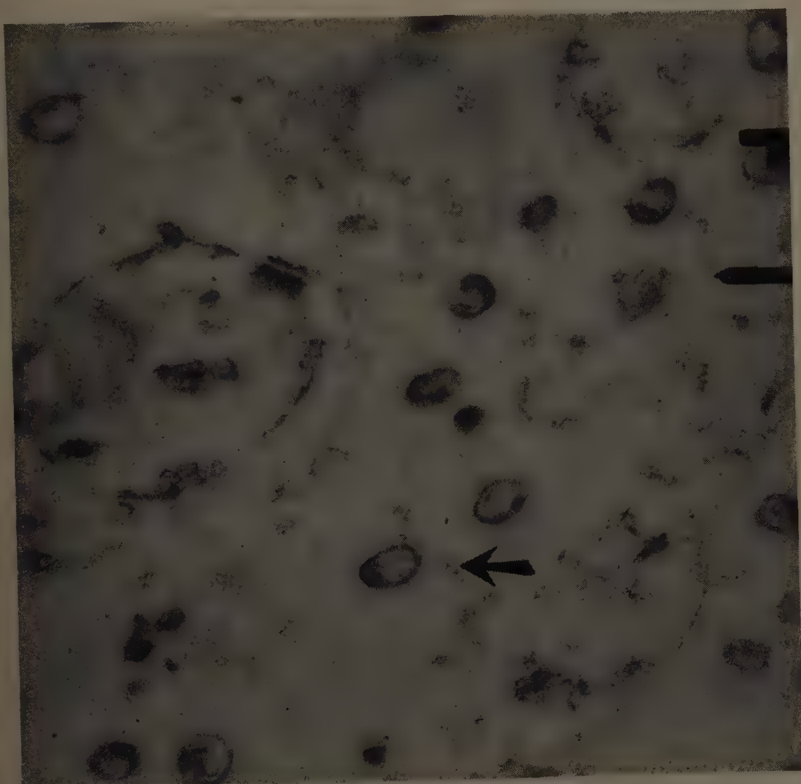

FIGURE 4. Electron photomicrograph of vaccinia particles in infected rabbit kidney cells. ×5000.

Isolation of the Monkey Pox Virus

The etiological agent of monkey pox has been isolated from naturally infected animals by using both embryonated chicken eggs[4] and tissue cultures[4,6] (TABLE 2). On the chorioallantoic membranes, grayish edematous changes with small discrete lesions were produced. The lesions showed a tendency to spread along the blood vessels, were consistently smaller than those resulting from vaccinia infection, and showed no evidence of hemorrhage.

The titers obtained on egg membranes from the material examined in the Danish outbreak[4] were as high as 10^{-8}, but in our experience[6] end point dilutions varied between 10^{-4} and 10^{-5}.

Virus from lesions of naturally affected animals has been isolated directly in cell cultures of rabbit kidney epithelium.[6] Forty-eight hours after inoculation the cell sheets were completely affected, although cytopathogenicity was detected as early as twelve hours. Affected cells were connected by threadlike cytoplasmic elongations and, as the disease progressed in the sheets, areas of cells sloughed from the glass, leaving islands of affected cells, until finally the entire sheet had slipped from the glass at seventy-two hours.

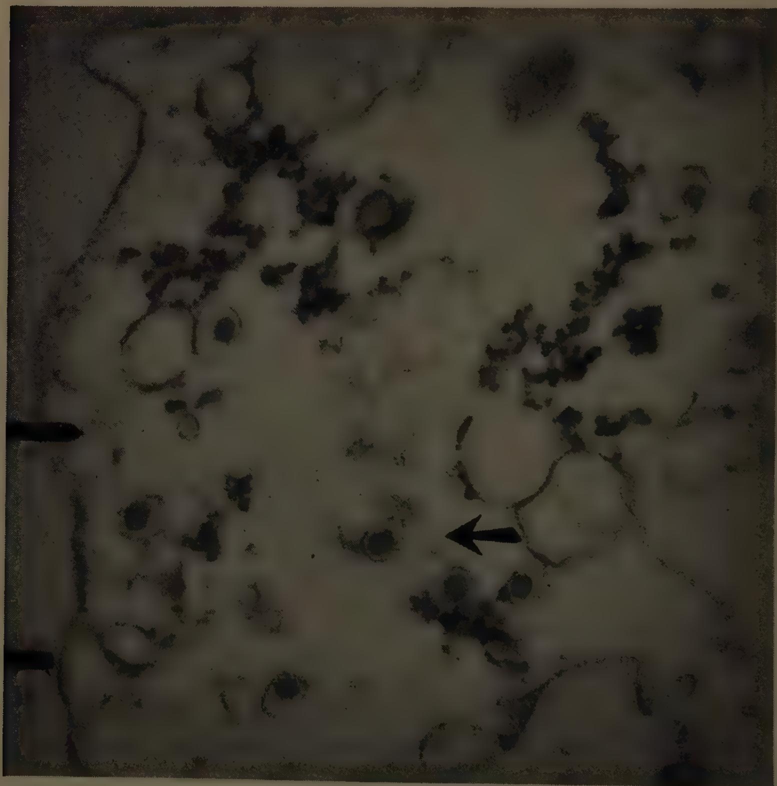

FIGURE 5. Electron photomicrograph of monkey pox virus particles in infected rabbit tissue culture cells. ×7500.

Microscopic examination of stained infected cell sheets reveals the presence of numerous cytoplasmic eosinophilic inclusion bodies (FIGURE 2). The bodies vary in size and number and are most frequently located close to the nuclear membrane. In some cases the inclusions cause indentation of the nuclear membrane, and often a clear halo is present around the individual bodies.

Virus Identification

The pathogenic characteristics of the agent described above clearly identify it as a pox virus, but further studies have been done to attempt positive identification of the virus with known pox viruses.

Lack of pathogenicity for the mouse by intraperitoneal injection eliminates the possibility of identification as ectromelia virus. Lesions on the chorioallantoic membrane typical of cowpox virus have not been seen, and antigenic differences in the diffusion-precipitation test of Gispen have been noted.[4]

The virus produces a hemagglutinin for chicken erythrocytes that is inactive against other mammalian red cells, and the hemagglutination is inhibited specifically by serum of monkeys that have recovered from infection. Furthermore, a rise in hemagglutination-inhibition antibody was demonstrated between acute and convalescent serum samples from infected monkeys. Cross-hemagglutination-inhibition tests on both monkey pox and vaccinia antisera show approximately equivalent titers.

Complement-fixation tests using both vaccinia and monkey pox antigens and antisera have indicated a definite relationship between the two agents, but differences between homologous and heterologous titers indicate that some antigenic dissimilarity exists.

Active immunity of mice was demonstrated with both monkey pox virus and vaccinia virus. Both agents were capable of protecting animals against challenge with monkey pox virus by the intracerebral route (TABLE 3).

It seems definite that the monkey pox virus is a member of the variola-vaccinia group. Whether it is distinct from known agents is still an open question. Certain characteristics tend to make consideration of the virus as a separate entity possible, but the real position of monkey pox virus in relation to previously identified pox virus yet is to be determined.

Morphology

The appearance of the virus particle has been studied in electron photomicrographs,[4] which show the virus to be rectangular, a feature similar to that of other pox viruses. Measurements of particles show the size range to be 200 to 250 μ (FIGURES 3, 4, and 5).

Summary

A pox disease that has appeared in laboratory monkeys in at least two parts of the world is reviewed. The virus responsible for the outbreaks is a member of the variola-vaccinia group and affects both *Macaca mulatta* and *M. philippinensis* (cynomolgus) species. Presently available information is insufficient to establish firmly the position of this newly described virus in relation to other members of the variola-vaccinia group.

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B VIRUS INFECTION IN MONKEYS

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B virus was first isolated by Sabin and Wright in 1932 from the central nervous system of a laboratory worker who had died after being bitten by a monkey.[1] For almost twenty-five years the symptoms in monkeys escaped clinical detection, usually because, when the virus was isolated in tissue culture, the monkey concerned either had died or had recovered from the infection.

It has become evident from antibody studies that the disease is widespread among rhesus monkeys. Thus, in random samples of 100 monkey sera taken in the laboratories of my associates and myself we found 17 with titers greater than 1:4; other authors reported antibody in as many as 100 per cent of monkeys tested.[2] Furthermore, when samples were taken over a period of time, it was possible to show a gradual increase in the number of positive sera, indicating that in any large colony one might expect to find clinical cases.

In 1956, during routine oral dosing with antibiotics, we noticed herpeslike ulcers on the lips and tongues of a number of rhesus monkeys (FIGURE 1). From these lesions we were able to isolate B virus in tissue culture.[3] The identification was based on the typical cytopathic effect in tissue culture, on neutralization with B antiserum, and on intracerebral inoculation of rabbits, which caused death on the fifth day. Typical intranuclear inclusions were seen histologically in monkey and rabbit tissues.

In order to establish Koch's postulates for the identification of a causal agent, we reisolated the virus from the rabbit brain and inoculated it into the tongue of an antibody-free monkey. Typical tongue lesions were produced four days later showing a histological structure identical with that of the natural cases.

The infection is of the greatest importance to monkey-house personnel, since there have been at least twelve authenticated fatal human infections, most of them in the last three or four years.

My purpose is to describe B virus infection as it occurred in our colony during 1956–58, first its incidence and then the clinical features and the gross and microscopic lesions.

Incidence

Clinical examination of 14,400 rhesus monkeys revealed 332 with tongue or lip lesions, an incidence of 2.3 per cent. These monkeys had been kept in communal cages in groups of 60; there is evidence that segregation in smaller groups, particularly in pairs or singly, reduces the incidence.

In our view, cynomolgus monkeys under natural conditions are probably free from this disease; certainly, no cases were seen among 6000 kept in isolation from rhesus monkeys. However, on one occasion several of the cynomolgus in contact with infected rhesus developed typical mouth lesions ac-

companied by severe systemic disturbances that sometimes resulted in death. Our inference concerning the causal agent in these cases is based on the histological finding of typical inclusions, since no virus isolation was attempted.

A seasonal variation has been noted in the number of cases diagnosed in our laboratory, the incidence being low in spring and highest around October.

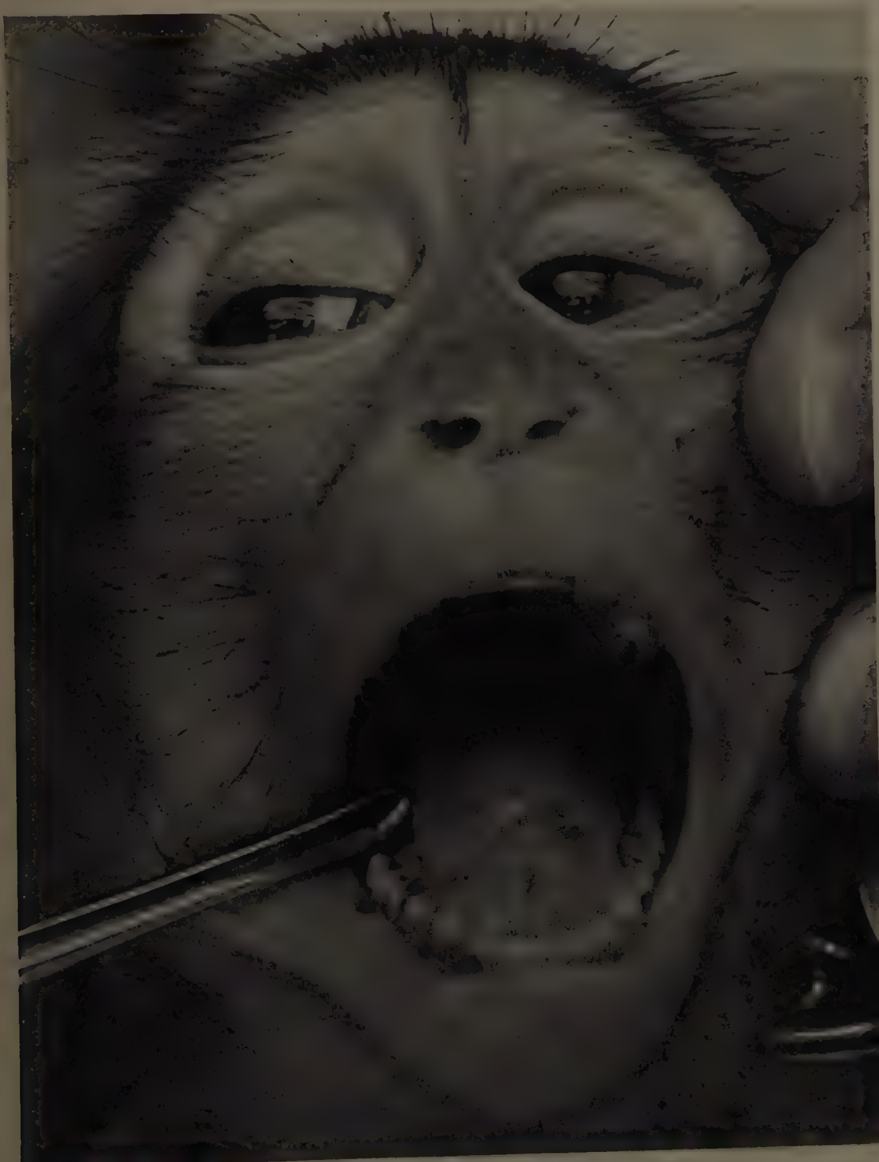

FIGURE 1. B virus lesions on the tongue and lower lip of a rhesus monkey. Reproduced by permission of the *Journal of Pathology and Bacteriology*.

It may be that the monsoon period has a bearing on this, since the attack rate nearly parallels the rainfall curve, with six weeks' delay: one might expect the animal population to be more susceptible to infections after the monsoon because of a lowered resistance.

Clinical Disease

In describing the clinical course and pathology of any disease causing relatively little systemic disturbance it is important to remember that indi-

FIGURE 2. An early lesion between the left circumvallate papillae; an ulcer at the tip of the tongue.

vidual variations from case to case will be much greater than those in acute fulminating infections. It is unlikely that any single monkey will be found to possess all types of B virus lesions fully developed; it is only by examining a large sample that it becomes possible to indicate the sites of localization of damage.

The most readily recognized gross lesions are found on the surfaces of the tongue (FIGURES 2 and 3) and buccal cavity and on the mucoepithelial border of the lips. These lesions initially consist of small vesicles, which soon rupture to give an ulcer that heals below a fibrinous necrotic scab. On the lips and skin this scab is brownish-red, dry, and dense, whereas in the buccal cavity the scab is a yellow-gray translucent plaque sharply demarcated from the normal tissues.

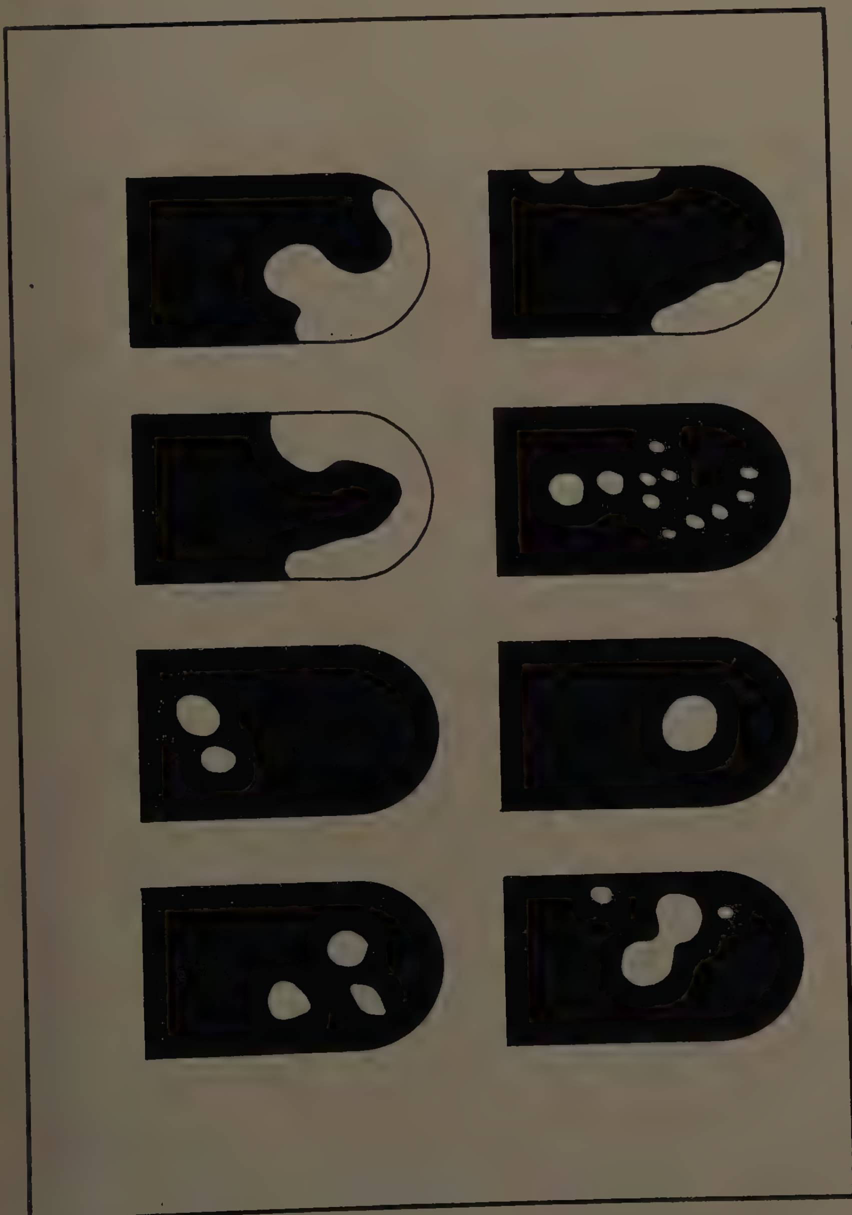

FIGURE 3. Typical distributions and relative sizes of tongue lesions.

Swabs taken in the early stage of vesicle or ulcer formation yield B virus in tissue culture. The lesions usually heal in from seven to fourteen days, and no visible scar remains, since the lower layers of the dermis are not necrosed.

The monkeys appear to suffer little inconvenience even when the ulceration is severe, and they continue to eat grain without much difficulty. It is difficult to recognize infected monkeys by their general appearance, since they usually behave normally; temperatures usually are elevated only slightly in the absence of any secondary infection. A slight mucopurulent nasal discharge is often present, but it disappears at about the tenth day. There is often a concurrent conjunctivitis of varying severity. We often have observed that the infected animals have diarrhea, but this is such a common occurrence in laboratory monkeys that it is of doubtful diagnostic significance.

Secondary infections of the buccal lesions are seen occasionally. Fungal infection will give rise to grayish rough lesions in which the hyphae penetrate into the necrotic tissues (FIGURE 4); when bacterial infection occurs, a slimy fetid discharge is present over the ulcers and around the lips (FIGURE 5), and the monkey then appears toxemic and is obviously distressed

The only other external lesions we have found have consisted of thick scabs on the body, ranging from 5 mm. to 2 cms. in diameter and as much as 5 mm. thick. Presumably these arise from infected bites or scratches; we have been unable to isolate B virus from them, but they can be differentiated from normal scabs by the finding of inclusion bodies on histological examination.

Histology

The earliest stage of vesicle formation is a ballooning of the cells of the malpighian layer of the epidermis, with leukocytic invasion of the area; spaces rapidly form by breakdown of the cells and, at this stage, fluid can be aspirated for virus isolation. The keratinized cells overlying the vesicle slough off, leaving a plaque of necrotic fibrinous material overlying the base of the ulcer. The necrosis seldom penetrates below the dermal papillae, but cellular infiltration into the muscles of the tongue may be seen in the severer cases. Healing is by the formation of granulation tissue that gradually replaces the plaque.

Intranuclear inclusions can be seen in the tissues showing the most recent signs of degeneration, that is, in the cells near the junction of plaque and normal tissue, where ballooning is taking place (FIGURE 6). The inclusions range from a finely granular, eosinophilic mass filling the nucleus to a condensed shrunken mass withdrawn from the nuclear borders into a typical type A inclusion. The cells containing such nuclei often aggregate into multinucleated masses that are quite distinctive.

Of the abdominal viscera the liver and kidneys most frequently show histological evidence of damage. In the liver this may be confined to an infiltration of leukocytes and monocytes around the vessels in the periportal connective tissue, without any inclusions; about 60 per cent of our cases show

lesions of this type. In about 5 per cent of cases the liver shows additional lesions in the form of necrotic foci in which the liver parenchyma is severely damaged; in these cells inclusions readily can be found.

The kidney cortex usually is more or less normal, but in some 75 per cent of cases there is leukocytic infiltration and, occasionally, necrosis of the tubular elements of the medulla. We have failed convincingly to demonstrate

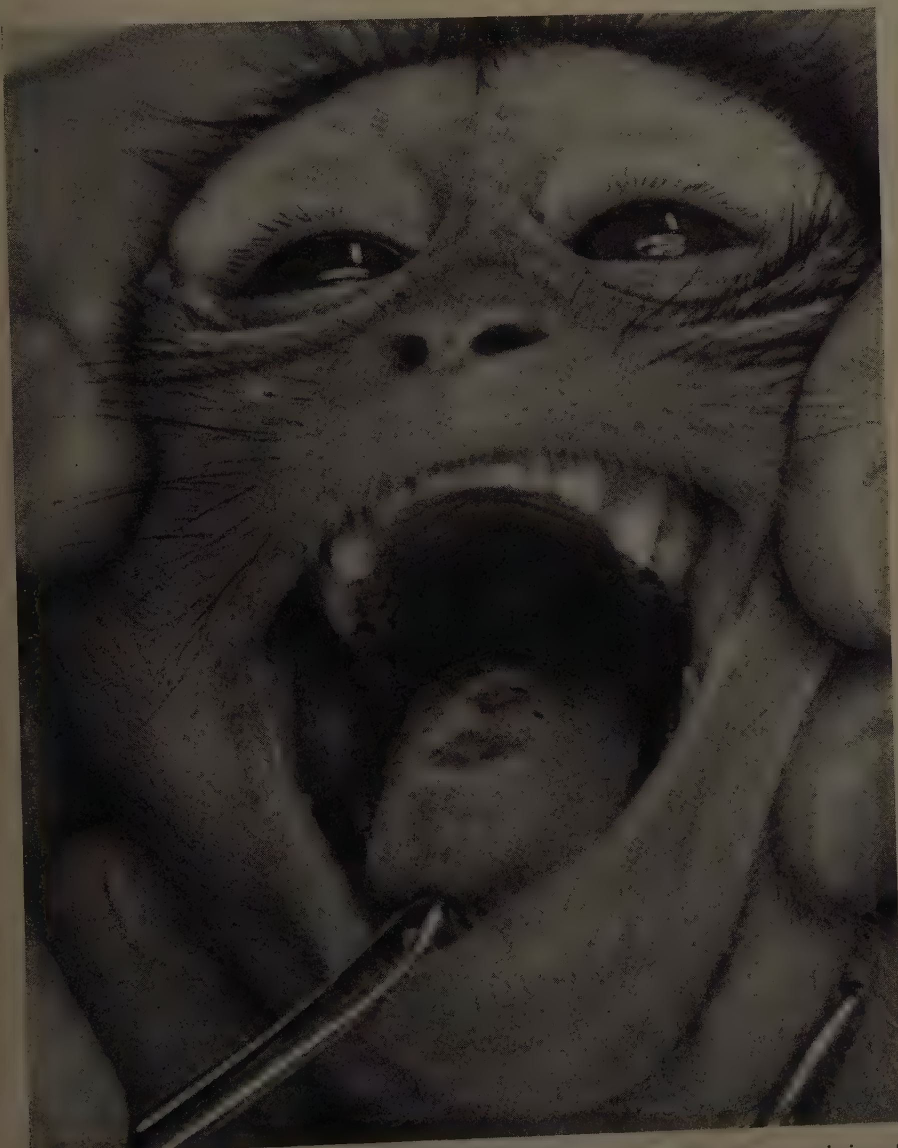

FIGURE 4. Tongue lesions with secondary fungal infection. Reproduced by permission of the *Journal of Pathology and Bacteriology.*

lesions in the lungs, although pneumonic areas of various ages have been seen.

Probably one of the most significant findings is that the adrenals of the monkeys thus far examined by us appear to be normal. This is in direct contrast to the pathology of the fatal disease in man and the rabbit, in which adrenal necrosis has been most marked. A comparison may be drawn here

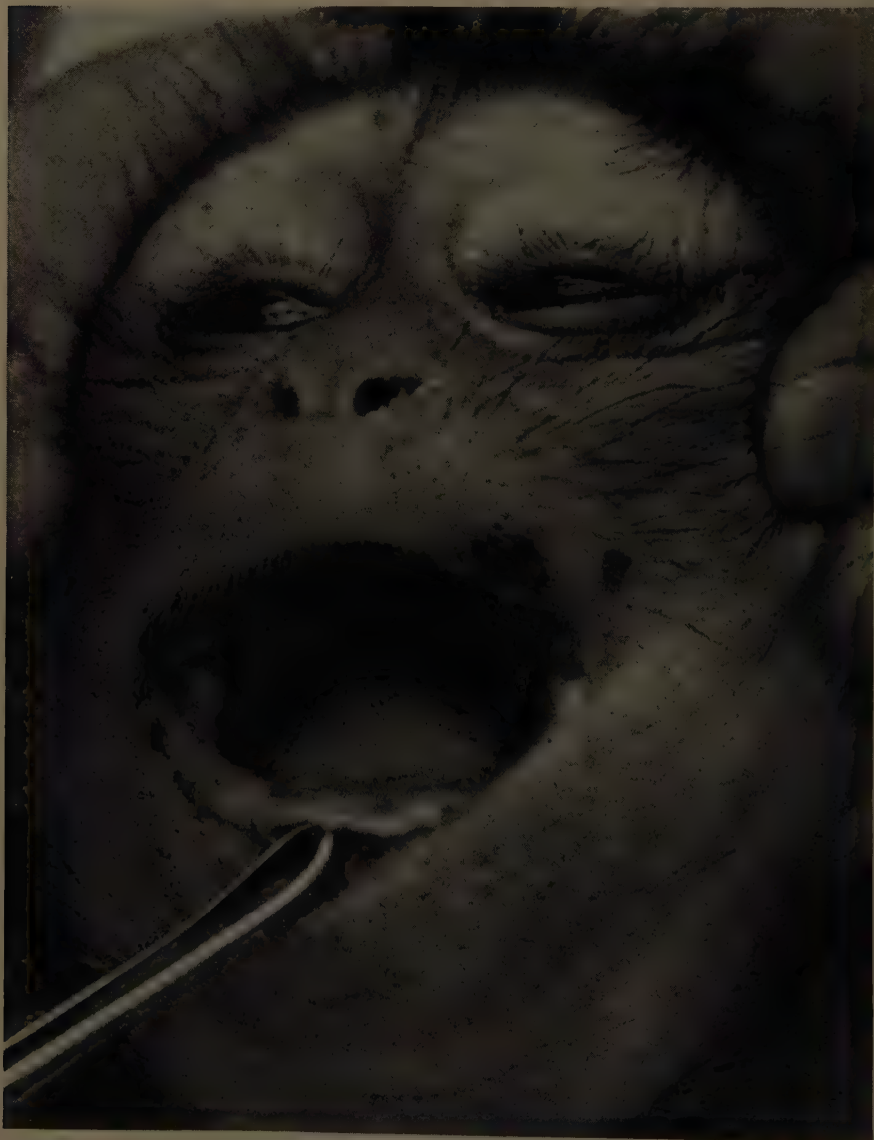

FIGURE 5. Necrosis of the tongue and right lower lip due to secondary bacterial infection of B virus lesions. Dry herpeslike ulcers are seen on the left upper lip.

with herpes virus, which causes acute adrenal necrosis in rabbits and in children dying from the disease, but apparently not in man with mild herpes infection, although information in this connection is not easily obtained.

It would seem possible that the degree of adrenal damage has some bearing on the severity of the symptoms or on the ultimate outcome of these diseases. It is relevant here to mention a suspected human case of B virus infection that caused ascending myelitis, in which the progress of the disease appeared to be checked by the administration of 200 mg. of cortisone daily. The patient subsequently recovered, but some paralysis remained.[4]

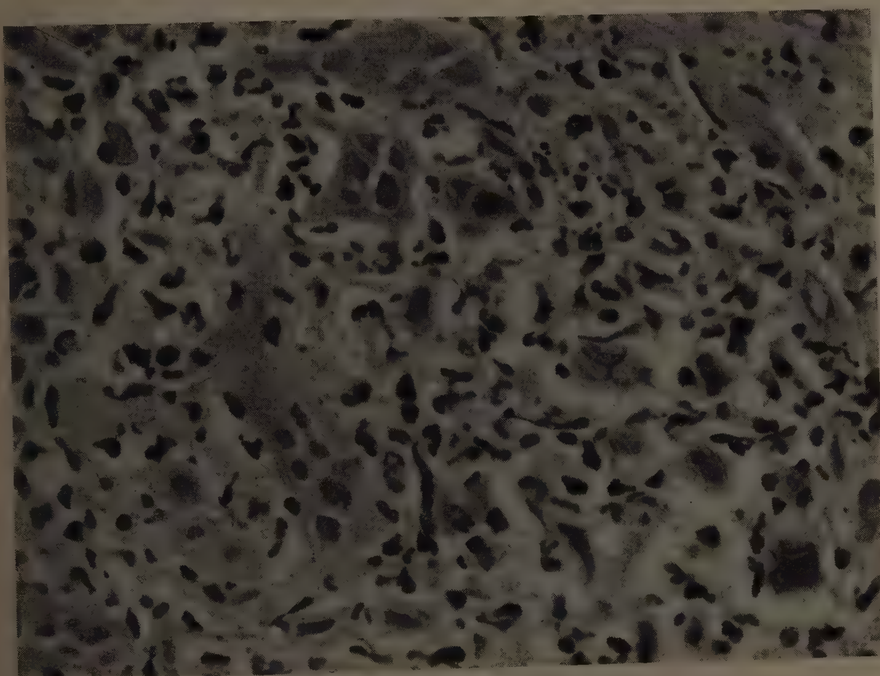

FIGURE 6. Inclusions seen in the cells at the periphery of an ulcer. ×750.

The central nervous system of infected monkeys shows typical lesions in the region of the pons and medulla (FIGURE 7). In 75 per cent of our cases we have seen vascular cuffing and microglial reactions in the region of the roots of the facial and trigeminal nerves. Lesions are seen also in the descending tract of the trigeminal nerve as it turns posteriorly between the strands of origin of the facial and auditory nerves. Similar lesions are also common around the tractus solitarius in the medulla. Since it is to these areas that sensory impulses pass from the tongue, probably the virus spread is either axonal or periaxonal and, if diffusion to other parts of the brain stem does occur, then those areas are incapable of response. Neuronal changes are not normally seen in the affected areas.

On one occasion we produced experimental lesions by inoculating the virus

below the tongue of an antibody-free monkey; typical ulcers were produced. When the animal was sacrificed on the tenth day, lesions were found in the pons and medulla that were identical with those seen in spontaneous cases. On the other hand, a monkey inoculated intracerebrally showed no histological lesions.

We have failed to find spinal cord lesions in these cases, except for one lumbar cord neuron in one monkey. One other monkey inoculated intradermally in the flank showed cytoplasmic vacuolation of neurons in the lumbar cord. There were no inclusions and no cell reaction; the significance of the lesions is therefore unknown.

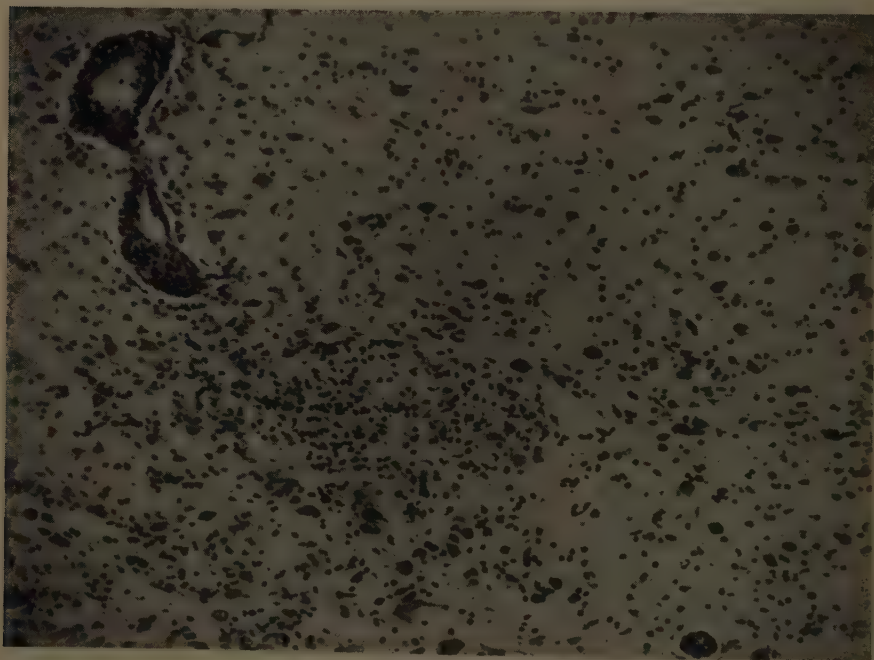

FIGURE 7. Glial reaction in the tractus solitaris of an infected monkey. ×150. Reproduced by permission of the *Journal of Pathology and Bacteriology*.

There are probably two routes of virus spread in B virus infection. Virus produced in the oral lesions passes along nerve roots directly into the brain stem, where it stimulates a mesodermal reaction. We have found, in the trigeminal nerve trunk, lesions that may have been due to virus ascending in this way. The other means of spread is the blood. Evidence that this type of spread occurs is given by the occasional isolation of the virus from sera of monkeys in the course of routine laboratory experiments. It is presumably by this route that the viscera become infected.

Virus spread within the colony is probably by the contamination of drinking water and food with saliva from infected animals, a secondary route being direct inoculation through scratches or bites.

It seems clear that this disease can be extremely common under certain laboratory conditions. The lesions are such that virus release readily occurs so that, although the monkey colony may show little in the way of symptoms, the laboratory personnel may be at considerable risk. Efforts must be made to prevent animal handlers from exposing themselves to this risk; not only are suitable gloves essential, but masks and goggles should be worn to prevent droplets entering the mouth, nose, or eyes. Equipment contaminated with saliva can be equally dangerous and should be treated with the utmost care.

There are many aspects of the condition still remaining to be clarified, but it is hoped that a knowledge of the clinical disease as seen in the monkey will enable rational precautions to be taken to protect laboratory personnel against this most serious hazard.

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B VIRUS INFECTION IN MAN

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B virus, herpes B, or *Herpesvirus simiae* may cause in man a viral encephalitis or encephalomyelitis, usually with fatal consequences. In monkeys, the disease may give a clinical picture similar to that produced by *H. hominus* in man.

The disease is rare, the first known case occurring in 1932 and reported by Sabin and Wright in 1934.[1] There is no record of another case until Sabin[2] in 1949 reported a second one. Thus the disease was almost completely unknown to the medical profession until recently. Standard clinical texts did not mention it, and only brief mention of the disease was made in more specialized texts.

It was only with the tremendous increase in the importation and use of the *Macaca mulatta* (rhesus) monkey and the *M. philippinensis* (cynomolgus) monkey for research and for biological production, in which monkey renal cells were used for tissue culture, that the importance of the disease in man and of its prevention became apparent. The total number of known cases is somewhat in doubt, but probably does not exceed fifteen. Twelve of them are reviewed in this paper. Nine occurred in 1957 and 1958 in England, Canada, and the United States.

Case Reports

The first case[1] was that of a 29-year-old physician engaged in a research project who was bitten on the ungloved fingers by an apparently normal rhesus monkey. The wounds were superficial and were treated with a topical antiseptic. Three days later pain, redness, and swelling at the site of the wounds developed and, during the next three days, lymphangitis and lymphadenitis of the involved upper extremity. The patient became febrile, acutely ill, and was hospitalized. Of special interest were several small herpetic vesicles around the wound. The patient then developed paralysis of the lower extremities, bladder retention, and a rapidly progressing, ascending myelitis, and died in coma of respiratory failure seventeen days after receiving the bite.

The autopsy revealed no remarkable gross lesions. The cord was somewhat edematous and the spleen was soft. There were several enlarged, moderately hemorrhagic lymph nodes in the axilla of the involved extremity. The histological picture of the mid-cervical and upper dorsal regions of the cord suggested a transverse myelitis. Areas of focal necrosis were noted in the regional lymph nodes, the spleen, and the adrenals. It was not possible to transmit the disease to the rhesus monkey, mice, guinea pigs, or dogs by the application of glycerinated specimens taken at autopsy, but the inoculation

of rabbits demonstrated a strongly neurotropic filterable virus in the patient's brain, cord, and spleen. A disease developed in the inoculated rabbits that strongly resembled the disease in man in the local lesions produced, the length of the incubation period, the development of urinary retention, flaccid paralysis of the lower extremities, and an ascending myelitis, with death by respiratory paralysis. Focal necrosis was also noted in the liver, spleen, and adrenals.

The second case was reported in 1949 by Sabin, who described the case of a 25-year-old physician who was engaged in research using the rhesus monkey. This case is interesting in that there was no history of a monkey bite. However, there was a history of the contamination of a fresh superficial wound on the right index finger by saliva from a stomach tube being removed from the animal. Within a few days a lesion was noted at the wound site. Progressively there developed lymphangitis, lymphadenitis of the involved extremity, a febrile illness, herpeslike lesions of the affected arm, axilla, and anterior chest, neurological involvement manifested by dysphagia, mental confusion, a lack of deep tendon reflexes of the lower extremities, inability to expel enema, urinary retention, and bilateral nystagmus. Death by respiratory paralysis followed.

An infectious agent proved to be B virus was recovered from the right axillary lymph node and from the central nervous system by the inoculation of rabbits. No virus was recovered from the spinal fluid, liver, or spleen. The virus was neutralized by specific B-virus antiserum.

It was not until 1957 that the third recognized case occurred. Hummeler *et al.*[3] reported the case of a 28-year-old animal attendant who became ill in March 1957. The patient's prodromal symptoms were generalized muscular aches and pains and an elevated temperature. For several days he was treated at home for a grippelike illness. He then was reported to have convulsions and a fever of 106° F. and was hospitalized. The referral history indicated that he had been lethargic for two days before hospitalization. Neurological examination revealed ataxia, a broad-based gait, no abdominal reflexes, and a positive Rhomberg. A right hemianesthesia to pinprick then developed.

A spinal fluid examination revealed an increased pressure of 440 mm. H_2O, and 160 cells/cc., 95 per cent of which were lymphocytes.

The patient became apneic, cyanotic, and comatose. He was placed in a respirator and a tracheotomy was performed. He devolped a complete flaccid paralysis of the left arm and leg and a partial flaccid paralysis of the right arm and leg. Consciousness returned, but the progress of the disease continued unabated. The patient developed anesthesia as far as the mandible, bilateral nystagmus, weakness of the facial muscles, deviation of the tongue to the right, and immobility of the palate. He then lost all motor power with the exception of the voluntary movement of his eyelids. He became comatose again and died two days later, thirty days after the onset of illness.

In this case there was a history of several monkey bites and scratches during his six months of employment as an animal attendant, the last one occurring five weeks before the onset of illness. No local lesions were noted.

At autopsy the gross examination revealed nothing remarkable. Microscopic examination revealed findings compatible with those of viral encephalitis involving the medulla, pons, thalamus, and cortex. No inclusion bodies were found.

No virus was isolated from specimens taken at autopsy. It was thought that the long period of time between onset of illness and death made the possibility of virus isolation remote.

Two spaced serums in the complement fixation test showed a rise in antibody titer against *H. hominus.* This fact was highly suggestive of an infection with *H. simiae* if *H. hominus* could be ruled out. It could not be, with the complement fixation test, but the neutralization test made the diagnosis possible.

The fourth case also occurred in March 1957 and was reported by Nagler and Klotz.[4] A 31-year-old veterinarian engaged in the control of poliomyelitis vaccines and occupied primarily with the inoculation and autopsies of rhesus monkeys developed a fatal B virus infection. His symptoms progressively were headache, dysphagia, unilateral hyperesthesia of face and scalp, diplopia, and vertigo. He became febrile and ataxic. After these symptoms he experienced spasms of the abdominal muscles and diaphragm, nuchal rigidity and mental confusion, and died seven days after the onset of illness.

In this case there was no reported history of monkey bite or other injuries related to his work.

Histological examination revealed extreme inflammatory and degenerative changes in the cord, particularly in the cervical area, that were not restricted to any specific spinal tracts. No inclusion bodies and no areas of focal necrosis of the viscera were found. B virus was isolated from the cord and brain stem.

Interestingly, the patient's serum contained neutralizing antibodies and complement-fixing antibodies to *H. hominus.* The sera taken before illness, during illness, and post-mortem did not vary in titer. Therefore, it seems likely that the presence of circulating antibodies to *H. hominus* offered no protection against a B virus infection.

The fifth case* was that of a 30-year-old chemist who was hospitalized in a semicomatose state in April 1957. Neurological examination revealed only an absence of abdominal reflexes on the right. The patient died in coma the following day. Investigation revealed that a week before hospitalization he had fallen, but suffered no apparent injury; he may have been ataxic at that time. Within a few days he had become drowsy, lethargic, nauseated, and had occasional hiccoughs.

His work history revealed that he had cleaned the skull of a rhesus monkey, without wearing gloves, two weeks before the onset of illness. However, there was no history of injury during this procedure.

A virus was isolated from the brain and was identified by the neutraliza tion test as B virus.

The sixth case,† occurring in July 1957, was that of a 19-year-old animal

* Personal communication from McNair Scott, University of Pennsylvania School of Medicine, and Children's Hospital of Philadelphia, Philadelphia, Pa.
† Personal communication from McNair Scott.

attendant who was bitten on the left index finger by a rhesus monkey. He did not report the injury until five days later, when the wound was grossly infected. It was treated and he was given 30 cc. of gamma globulin intramuscularly. Thirteen days later he complained of severe headache and weakness and was hospitalized with the diagnosis of encephalitis. The only other neurological finding was absence of abdominal reflexes. His temperature rose to 104° F. and came down by lysis in two days. He then developed diplopia and the muscular weakness increased. He became comatose and died eight days after onset of illness and twenty-six days following the monkey bite.

A virus was isolated from the brain and identified as B virus.

The seventh case[5] is one of a patient who has survived a B virus encephalomyelitis. The patient, a 31-year-old animal attendant, was bitten twice in one day, by a rhesus and a cynomolgus, in September 1957. Both wounds healed normally, with no vesicle lesions developing. Two weeks later he complained of pain between the shoulder blades and five days after that, a slight fever and headache. While cycling to work the next day he noticed a weakness of the left leg and returned home. The following day the weakness of the left leg increased and he suffered urinary retention. He was hospitalized and, within two days, all four extremities were paralyzed, he was unable to swallow, and his respiratory efforts were affected, so that both a tracheotomy and a respirator were necessitated. His temperature remained moderately elevated and, within two days, he was in a serious condition. Cortisone, 100 mg. intravenously and then 200 mg. intramuscularly, was given daily. Within two days his swallowing reflex began to return and his temperature gradually returned to normal. During the next five weeks his illness was complicated by a collapse of the left lung, a pressure ulcer, and a urinary tract infection. By mid-November it was possible to remove him from the respirator. He improved gradually until by March 1958 he had regained the use of all extremities except for some weakness of the left leg and was able to walk short distances unaided. He appeared mentally normal, and it was expected that his recovery would continue so that he could return to work. Sera taken on the third, eighth, twenty-sixth, and hundredth days and tested for B virus antibodies gave titers of 1:16, 1:25, 1:48, and 1:32. On the basis of the rise in antibody titer to B virus, a B virus encephalomyelitis was suspected.

This case is noteworthy for two reasons. First, it is one of two known cases of survival and second, the onset of recovery coincided with the administration of cortisone.

The eighth case,[6] occurring in November 1957, was reported by Pierce *et al.* The patient, a 34-year-old animal attendant, was ill for only two days and then died. On the day preceding his death he complained of diplopia and was said to have been agitated and delirious later that day. The following day, after visiting his doctor, he stated he felt much improved. He took a nap and shortly thereafter was found dead.

There was no history of a monkey bite for four months preceding his illness. His work for the seven weeks preceding his illness was that of caring for normal monkeys.

A virus was isolated from the patient's brain and cord and identified as B virus. The autopsy findings were compatible with death due to respiratory failure and inclusion body encephalitis.

The ninth case,[3] reported by Hummeler *et al.*, was that of a 21-year-old laboratory technician engaged in poliomyelitis vaccine production in January 1958. His accident record revealed that on two occasions he had suffered minor lacerations on his hands from broken glass containing tissue cultures of monkey renal cells. The second injury was incurred two days before the onset of his illness. His prodromal symptoms were those of an upper respiratory infection. In addition, there was noted an infected large blister on his left thumb. He was treated in the plant dispensary and referred to his personal physician. He had visited his personal physician the previous evening, the blister had been treated, penicillin given intramuscularly, and oral penicillin and sulfa therapy initiated. An enlarged axillary node was noted. In addition, medication was given for his cough, and it was noted that he had complained of a persistent cough for approximately five weeks. He felt much improved, but six days later complained of chills and stiffness of the neck. Two days later his temperature was slightly elevated and, two days later, he complained of difficulty in swallowing. Neurological examination revealed nothing except diminished patellar reflexes. He was hospitalized for observation. Shortly thereafter diplopia and dizziness developed and he stated that he needed water to help him swallow solid foods. Spinal fluid examination revealed a pressure of 248 mm. H_2O and 157 cells/cc., 97 per cent of which were lymphocytes.

During the next two days he suffered increasing muscular weakness, inability to swallow, dyspnea, and restlessness. He was placed in a respirator and a tracheotomy was performed. His temperature was 105° F. His condition appeared to improve, he could move all extremities, he was mentally clear, he could swallow sips of water without difficulty, his diplopia was clearing, and he was able to be out of the respirator for short periods. However, he suddenly became cyanotic and died.

Histological examination revealed a diffuse encephalitis compatible with a diagnosis of viral infection. No inclusion bodies were found. B virus was isolated from the central nervous system and was identified by the neutralization test.

This is the only reported case in which there was no direct contact with monkeys; the exposure was only to tissue cultures of monkey renal cells.

The tenth* case occurred in February 1958. A 29-year-old animal attendant at first complained of fatigue, headache, and epigastric pain. The tentative diagnosis was perforating peptic ulcer. One week later he had difficulty in micturition. This symptom was followed rapidly by paralysis of the legs. A rapidly progressing, ascending myelitis ended in his death the following day. B virus was isolated from his central nervous system and identified by the neutralization test.

There was a history of a puncture from a needle that may have been used previously to inject monkeys, an accident that occurred five days before his illness.

* Personal communication from McNair Scott.

The eleventh case* was that of a 40-year-old animal attendant who became ill in March 1958. His accident record indicated that five weeks before the onset of illness he was scratched on the hand by a needle used for injecting monkeys and, four weeks prior to his illness, he was bitten by a monkey. Both wounds healed normally. His initial symptoms were headache and nausea. Because of his type of work and accident history the possibility of a B virus infection was considered. No signs of neurological involvement were evident. Three days later the patient became acutely ill with a temperature of 105° F. and was hospitalized. He devolped a bilateral nystagmus, experienced an epileptiform seizure, became comatose, and died two hours after admission to the hospital.

B virus was isolated from the medulla and cord. Serum sample taken before the illness neutralized *H. hominus*, but had no neutralizing effect on B virus.

In addition to these cases there is one reported to have occurred in January 1956.* A 29-year-old animal attendant became ill. His history revealed that two days prior to the onset of illness he was scratched by a monkey. His illness began with a sore throat that was followed in two days by a fever of 105° F., headaches, and drowsiness. He was hospitalized. The fever subsided during the next week. He then developed left facial weakness, left hemiparesis, retinal detachment in the right eye, and mental deterioration. Five months later he was transferred to a mental hospital where he gradually improved during the next three years. The remaining symptoms are almost complete blindness in the right eye and diminished vision of the left eye. His mental state improved sufficiently to allow him to perform some tasks within the hospital and to make periodic visits home.

Neutralization tests on sera made before the illness showed a high titer against *H. hominus* and none against B virus. Sera taken after the acute phase of the illness neutralized B virus. Thus the results supported a diagnosis of B-virus infection. This is one of two cases of survival of a B virus infection.

Discussion

We have noted that, of the twelve cases briefly described, ten were of fatal encephalitis or encephalomyelitis, and that the two cases of survival involve severe residual central nervous system damage (however, one of these may make a nearly total recovery). In all but one case there are histories of direct contact with monkeys, and in that one case a history of contact with tissue cultures of monkey renal cells. In some of the cases there are specific histories of exposure by injuries and in others there is no history of injuries. In some, notably the first two cases, there are histories of direct exposure of wounds to contamination with monkey saliva. In many of the other cases the injuries reported may or may not be significant. Furthermore, there may have been numerous unrecognized minor injuries resulting in exposure. It seems to us that, in consideration of the several thousands of people who have handled monkeys and their tissues and the rather large percentage of monkey sera

* Personal communication from McNair Scott.

showing measurable titers to B virus, the susceptibility of man to a clinical B virus infection is low and that mere association with monkeys does not constitute exposure. The number of cases, few compared with the number of man-days of contact with the animals and their tissues and the number of injuries from monkey bites and scratches, as well as of other wounds from cages, equipment, needles, scalpels, and glassware, seems to favor man's low susceptibility to a B virus infection. The only definite thing that can be said in reference to exposure is that exposure to B virus requires direct contact with monkeys harboring B virus or with their tissues. The actual mode of transmission of the virus to man and the conditions required for this transmission are not well understood.

The diagnosis of a B virus infection in man can be accurately made only in a virus laboratory. However, since the symptomatology may vary a great deal, any encephalitis or encephalomyelitis developing in a person exposed to monkeys or their tissues should be suspected of being due to B virus infection until proved otherwise.

The laboratory diagnosis depends essentially on (1) the demonstration of a rise in antibody titer to B virus in the patient's blood during the course of the illness, (2) the isolation of the virus from the tissues and secretions and, sometimes, (3) the histopathological changes in the nuclei of affected cells.

These three diagnostic points are now discussed:

(1) In serologic examinations the test of choice is the neutralization test. B virus shares antigenic components with other members of the herpes group of viruses. Thus the complement test is unsuitable, especially since it does not distinguish between B virus and *H. hominus* infections. Neutralizing antibodies developing during a B virus infection do not reach high titers and usually are not discernible until two weeks after the onset of illness. Sera from acute and convalescent cases should be tested against B virus, preferably in rabbit kidney cell cultures.

(2) For virus isolation there is a variety of experimental hosts susceptible to infection with the B virus. The virus can be transmitted readily to rabbits by almost any route, usually with fatal consequences. Mice under three weeks of age are susceptible, especially suckling mice. This is in contrast to the case of *H. hominus* which also will infect older mice. The chorioallantoic membrane of embryonated chicken eggs may be used. The B virus produces pocklike lesions that are morphologically similar to those of *H. hominus*. The following tissue cultures have been used for B virus isolation: rabbit kidney, HeLa cells, monkey kidney, and human amnion. Of these, rabbit kidney cells are the most susceptible. Tissue specimens suspected of harboring the virus are better preserved in 50 per cent glycerol than by freezing.

(3) In histopathology: in experimentally produced infections, intranucleus inclusion bodies are formed in affected cells and are readily demonstrable. Their demonstration in patients who have succumbed to a B virus infection is most difficult, as it depends on the time elapsed between the onset of infection and death: inclusion bodies have been found in persons with fulminating encephalitis who died within a few days, but rarely in persons dying

after a prolonged ascending encephalomyelitis. The inclusion bodies morphologically resemble those found in *H. hominus* infections.

Safety Measures

Since it is known that the virus *H. simiae* can cause a fatal infection in man, and since at the present time there is no known method of treatment or of immunologic protection, it is mandatory that any institution housing or using monkeys, especially *M. mulatta* or *M. philippinensis*, adopt a comprehensive safety and medical program. Such a program must be carefully tailored to individual needs and circumstances.

To this end the following points shuld be considered.

. Animal quarters and laboratories should lend themselves to isolation techniques. They should be restricted to authorized personnel and to employees required to be in areas of possible exposure. The work areas should be large enough to permit safe working conditions. They should be light and designed for easy cleaning and disinfecting.

All employees required to enter areas of exposure should be thoroughly trained. Their training should include a review of the disease and an assurance that, if all safety and medical precautions are followed, the risk of infection by B virus is extremely remote. It is important that the training be given by "line" supervisors as well as safety and medical specialists, and that new employees or employees transferred into these areas should not be overlooked. Thus a meticulous checking system should be employed. The training should be broad and should be repeated periodically.

All safety procedures are based on the principle of isolation of persons from the virus. Thus, if we consider a person as having a protective envelope, the skin, perforated by several natural openings—in this case, the eyes, nose, and mouth—then the recommended procedures are sensible. They may be molded to meet individual needs. Obviously, careful laboratory methods and good housekeeping are essential.

The caging should allow for simplicity in capturing the animals. No animal should be caught by hand, as there has not been devised a hand-catching technique that in all cases will prevent an animal from biting the attendant. Furthermore, there are no gloves that allow for dexterity and yet protect against the opposing canine teeth of the monkey. Animal catching can be done by means of nets, boxes, confining tunnels, and stick and chain techniques. Inhalation or injectable anesthetics may be used under special circumstances. The principle involved is that the required task be performed in a manner that eliminates the possibility of the monkey's biting or scratching the attendant. Protective clothing for animal attendants should include complete skin coverage except for the face, which should be protected by a wire or plastic shield, and the eyes by a plastic shield or goggles. A heavy-duty coverall, boots, leather arm guards from wrist to shoulder, and heavy-duty gloves reinforced with wire mesh, staples, or metal studs should be used.

Each employee should have his own safety clothing, and dress areas should be provided so that he may undress, shower, and don his safety clothing, and then reverse the procedure before leaving the work area. A disinfectant foot bath also should be provided. A suitable mask should be provided,

especially for cage-cleaning. Every effort should be made to prevent breaks in the skin from cages and other equipment. All material, especially excrement, leaving the animal quarters should be autoclaved or put in plastic bags and incinerated.

Autopsies and dissection should be performed under generally recognized surgical conditions. All equipment and instruments used should be considered contaminated and must be disinfected or autoclaved immediately after use.

All monkey tissue and tissue cultures should be considered dangerous. Thus the necessity for protective clothing, including gloves and masks, obtains throughout all the work, as does the need for disinfection and autoclaving of all equipment.

In general, every attempt should be made to minimize the use of aerosols, especially those of a gross nature.

Every employee should be instructed to keep foremost in mind the importance of preventing breaks in the skin. Each should be instructed that, if he is injured while working, regardless of the cause of his injury, he should immediately wash the wound for at least three minutes with soap and copious quantities of water, apply a suitable topical antiseptic, and then proceed to the dispensary. The wound should again be cleansed with soap and water in the dispensary, a topical antiseptic applied, and the wound dressed. Moreover, an employee suffering a wound while off the job should report to the dispensary so that the wound may be treated and properly dressed before permission is granted to enter the work area.

The supervisor should be charged with the responsibility of enforcing all safety and medical procedures and of daily training of his personnel. He should be constantly on the lookout for methods of reducing accident potential.

Summary

There have been approximately fifteen cases of encephalitis or encephalomyelitis, usually fatal, in man, caused by B virus. All have been contracted by persons working in close contact with rhesus or cynomolgus monkeys, except for one instance in which the contact was with tissue culture of renal cells of these animals. However, this fact does not preclude in any way a danger from other species of monkeys. The number of cases has been small compared to the amount of exposure. In addition, from a review of the cases, it is known that the disease varies a great deal in symptomatology and that, once established, treatment is entirely supportive. The exact mode of transmission is not known, but evidence seems to implicate contamination of wounds and gross aerosols. The average incubation period is not known, nor whether subclinical cases occur, nor is the question of latent activity of the virus in man answered. Still to be solved are the problems of elimination of the disease in monkeys, as well as of definitive treatment and immunological protection for man. However, a carefully designed safety and medical program maintained daily and punctiliously is a realistic offer of protection for those required to work in contact with monkeys.

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EXPERIMENTAL IMMUNIZATION AGAINST B VIRUS

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The term "immunization" has been used, not with the inference that immunity has been produced successfully, but with its general meaning, which includes studies of both active and passive immunity. The objective of our attempts to produce immunity with inactivated vaccine is the immunization of monkeys. The problems involved in assuring safety from residual live virus are, in our opinion, too great to consider the use of vaccine in humans at this stage. It was first necessary, in any case, to demonstrate protection against infection in animals. This we have not yet done with practical success. The objective of our experiments on passive immunization is the use of immune monkey gamma globulin for prophylaxis in humans after exposure. Since protection can be studied in animals only, expectation of effectiveness in humans can be held by inference only.

The presence of B virus antibody in the serum of a normal monkey, the specificity of this antibody, and its relationship to herpes simplex virus antibody were first described by Sabin,[1,2] who thus was able to explain the difficulty encountered by Gay and Holden[3] in transmitting the B virus to rhesus monkeys and their observation that the sera of a large proportion of rhesus monkeys contained herpeticidal antibody. Sabin showed also that inoculation of monkeys with B virus gave rise to specific antibody and immunity to reinfection by various routes. Similar observations were reported by Burnet *et al.*,[4] who found that antibody resulting from B virus infection in monkeys neutralized herpes virus in equal or higher titer. Others, notably Hurst,[5] Melnick and Banker,[6] and Krech and Lewis,[7] have found B virus antibody in the sera of normal rhesus and cynomolgus monkeys. An increase in B virus antibody titer was noted by Keeble *et al.*[8] in a monkey with mouth lesions, which they have described as the naturally occurring syndrome. These various observations, which indicate that monkeys with antibody from natural or experimental infection are resistant to reinfection, gave a basis for attempting to produce immunity by vaccine or immune serum.

Additional evidence of resistance to reinfection of monkeys that have B virus antibody is given by the following 2 experiments in which the virus was inoculated by the intracutaneous route. This route was found by Sabin[2] to give rise to a specific local lesion. Cynomolgus monkeys were isolated in groups of 5, tested for B virus antibody, and retained if all in the group were negative. A second blood sample was taken immediately before inoculation of B virus. Serial tenfold dilutions of virus were injected at each dilution into monkeys in groups of 3. The animals were observed for 28 days for the development of skin lesions. Blood samples were tested for viremia on days 3, 7, and 14, and for antibody on days 14 and 28. The results are shown in TABLE 1. Every monkey, with the exception of 1, whose serum was found to contain antibody prior to virus inoculation, developed a lesion at the site of injection. The number of virus isolations from the blood was small, but

it is of interest that, when viremia was detected, it had occurred before the lesion appeared and probably before antibody was present in detectable amount. The failure to demonstrate viremia in monkeys given the larger

TABLE 1

LOCAL LESION, VIREMIA, AND ANTIBODY IN MONKEYS INOCULATED INTRADERMALLY WITH B VIRUS

Dilution* injected (0.2 ml.)	Monkey no.	Day local lesion appeared	Viremia on days 3, 7, 14	Antibody	
				Before	After
10^{-2}	C-291	7	— — —	—	+
	C-292	7	— — —	—	+
	C-294	7	— — —	—	+
10^{-3}	C-295	11	— — —	—	+
	C-306	—	— — —	+	+
	C-307	11	— — —	—	+
10^{-4}	C-301	11	+ — —	—	+
	C-302	11	+ — —	—	+
	C-303	11	— + —	—	
10^{-5}	C-308	11	— — —	—	+
	C-309	11	— + —	—	+
	C-310	13	— — —	—	+

* Titer of virus fluid 10^{-6} TCD_{50}/0.5 ml. in monkey kidney tissue culture.

TABLE 2

LOCAL LESION IN MONKEYS WITH AND WITHOUT ANTIBODY, INOCULATED INTRADERMALLY* WITH B VIRUS

Previous inoculation	Antibody	Monkey no.	Day local lesion appeared
B virus, intradermally, 6 weeks earlier; local lesion healed	+	C-292	—
		C-295	—
None	+	C-296	—
		C-300	—
None	—	C-299	7
		C-320	7

* Virus 0.2 ml. of 10^{-2} dilution. Titer of virus fluid 10^{-6} TCD_{50}/0.5 ml. in monkey kidney tissue culture.

doses of virus suggests that viremia may occur before 3 days if a large dose is given.

Six weeks later 2 monkeys with healed lesions, 2 monkeys found to have natural antibody, and 2 without antibody were inoculated with B virus. The results, shown in TABLE 2, confirm the observation that monkeys with antibody are resistant to reinfection. In a recent review Tobin[9] makes this statement: "Unlike herpes simplex infection in man, there is no evidence

that the [B] virus persists in monkeys in an infectious form after the initial lesions have healed." Our findings on the occurrence of B virus in kidneys and other organs would support this statement.

Experiments on Active Immunization

Preparation of vaccine. Since B virus is inactivated rapidly[7,10] in 1:4000 formalin at 37° C., lower temperatures were tested. After determining the rate of inactivation at several different concentrations and temperatures and preparing vaccines under various conditions, the following method was adopted. Virus fluids harvested from rabbit kidney tissue cultures, with virus concentrations of between 10^7 and 10^8 TCD_{50}/0.5 ml., were clarified by passing them through a coarse glass filter (sterilizing filters caused serious loss of virus) and then were inactivated in 1:1000 formalin at 20° C. for 72 hours. Since potency rather than safety—in terms of low probability of residual live virus—was the objective, inactivation was stopped when the concentration of live virus was estimated to be about 10^{-2} TCD_{50}/0.5 ml. Samples were taken for virus assay at 0, 24, 48, and 72 hours. The last sample, 50 ml. in volume, was inoculated into 100 roller tubes. Of 5 vaccines, only 1 contained virus (1 per 100 tubes positive) in the final 50-ml. sample. The rate of inactivation decreased during the 72-hour period. All virus assays and serum neutralization tests, except those shown in TABLES 1 and 2, were done on rabbit kidney tissue culture.

TABLE 3
EFFECT OF VACCINE IN RABBITS
Multiple* Intravenous Doses

	No.	B virus antibody titer†	Challenged with 50 TCD_{50} B virus intracutaneously			
			No. died	Days to skin lesion	Days to paralysis	Days to death
Vaccinated	6	>1:64 (5) 1:8 (1)	0	—	—	—
Controls	2	<1:4	2	6 to 8	10 to 11	12

* Three by 2 ml. per week.
† At time of challenge on twenty-eighth day. Tested against 200 TCD_{50}.

Antibody response and protection. Antibody response was irregular and varied in different lots similarly processed, suggesting lack of stability as a cause of this difficulty. Preliminary vaccines, made in the course of studying the method of preparation, gave only low levels of antibody in a small portion of rabbits given 3 doses of 2 ml. over a period of 3 weeks. Multiple doses of the last vaccine lot, BV8, produced high levels of antibody and immunity to a challenge dose of virus in rabbits, as shown in TABLE 3, but in monkeys 3 doses of this vaccine given over a period of 4 weeks produced neither significant antibody response nor protection against infection by intracutaneous inoculation. This vaccine was of too low a potency to be of any practicable value.

An earlier lot of vaccine, BV4, produced an antibody response in monkeys. In one trial, 120 rhesus monkeys were given 3 doses of 2 ml. 1 week apart. Thirty vaccinated monkeys and 30 controls were housed in each of 4 large cages. Vaccinated and control animals thus mixed freely and were handled similarly in all respects so that exposure to natural infection was equal in the 2 groups. Monkeys becoming ill in either a vaccinated or control group were removed, together with a monkey from the other group. The numbers in each group were thus kept equal. In order to reduce hazard from B virus exposure in the testing of large numbers of sera, herpes virus was used in the neutralization tests. The results of this trial are shown in TABLE 4. These data indicate that a secondary response was induced and, probably, a primary response, since the number of animals without detectable antibody was considerably lower among the vaccinated monkeys than among the controls. However, definite conclusions may not be reached from this type of trial.

TABLE 4
COMPARISON OF ANTIBODY TITERS IN VACCINATED AND CONTROL MONKEYS
30 Vaccinated, 30 Controls, in Each Cage

	Antibody* titer† (No. of monkeys)				Not tested	Total
	>1:4	1:4 to 1:16	1:16 to 1:64	>1:64		
Vaccinated (2 ml. at 0, 7, 14 days)	4	19	44	46	(7)	120
Controls	30	28	38	17	(7)	120

* Blood samples taken 3 to 4 weeks after third dose.
† Tested against 30 to 60 TCD_{50} herpes virus.

The antibody response in 2 rhesus monkeys without initial antibody given 2 doses of vaccine (lot BV5) and kept throughout the experiment in single cages is shown in FIGURE 1. Antibody response to vaccine is evident, but the levels are not high.

The same lot of vaccine (BV5) was used about 4 months later in a trial of protection against infection. Three monkeys tested for absence of antibody and isolated in single cages were given 2 ml. of vaccine 3 weeks apart and challenged by intracutaneous inoculation with B virus 1 week later, together with controls. The results are given in TABLE 5. The antibody response was poor. No definite evidence of protection was seen. However, since 1 vaccinated monkey did not develop a lesion and the other 2 had slightly longer incubation periods than had the controls, some degree of immunity may have been produced. It seems likely that this vaccine lost potency in the 4-month interval between the 2 experiments. These observations suggest that a more potent and more stable vaccine would give a definite degree of protection against infection by the cutaneous route. Whether or not such a vaccine would prevent the development of mouth lesions and natural spread of the virus could be answered only by further trials of suitable design.

Experiments on Passive Immunization

Preparation of immune monkey gamma globulin. Rhesus or cynomolgus monkeys were bled by heart puncture. The sera were screened for neutralizing antibody against herpes virus. Sera with titers of 1:100 or more against 60 to 100 TCD_{50} were pooled for concentration. This level included some 6 per cent of the sera tested, of which approximately one half showed detectable neutralization. A gamma globulin concentrate was prepared by

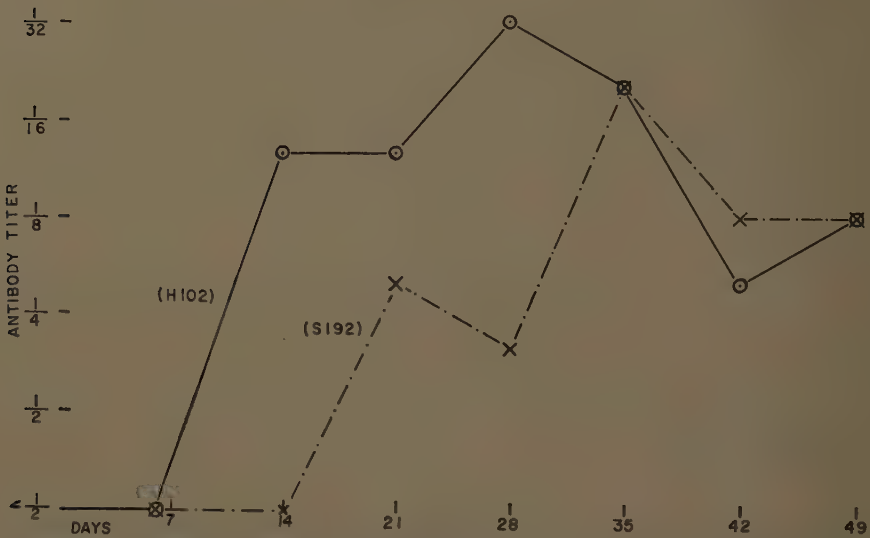

FIGURE 1. Antibody response in 2 monkeys given 1 ml. vaccine on days 0, 7, and 28. Sera tested against 100 TCD_{50} herpes virus.

TABLE 5
EFFECT OF VACCINE IN MONKEYS
2 Doses,* Interval 3 Weeks; 100 TCD_{50} Virus Intracutaneously 1 Week After Vaccination

Monkey no.	Antibody titer†		Local lesion after B virus inocul. (days)
	Before vacc.	After vacc.	
Vaccinated			
G-151	<1:4	1:8	10
H-780	<1:4	1:8	—
K-851	<1:4	1:8	10
Controls			
K-850	<1:4	<1:4	6
G-376	<1:4	<1:4	6
K-848	<1:4	<1:4	4
Pos. antibody			
G-578	>1:256		—
G-449	1:128		—
G-456	1:64		—

* First dose 4 ml., second dose 2 ml.
† Tested against 60 TCD_{50} herpes virus.

ammonium sulfate fractionation. A sterilizing filtration was carried out at the beginning and at the end of this process. The volume of the concentrated fraction was about one seventh of the volume of the serum pool. The titer of B virus antibody was increased fivefold, from approximately 1:25 to 1:125. The gamma globulin was tested for B virus and other simian agents by inoculation of rabbit and monkey kidney tissue cultures and of rabbits. The presence of these viruses seemed unlikely, since the concentrate neutralized 5 common simian agents and also was given 2 filtrations. Safety may be assured by holding the temperature at 37° C., at which neutralizing activity is undiminished after 6 months. The material was tested for toxicity by inoculation of mice, rabbits, monkeys, and 1 human subject.

TABLE 6
PROTECTIVE EFFECT OF MONKEY GAMMA GLOBULIN IN RABBITS
Intramuscular, 5 ml.

Challenge dose of B virus (TCD_{50})	Proportion died		
	Control	G.G. after 5 hours	G.G. after 1 hour
100	3/4	3/4	2/4
10	3/4	3/4	0/4
1	2/4	0/4	0/4

TABLE 7
PROTECTIVE EFFECT OF GAMMA GLOBULIN IN RABBITS
Intracutaneously, 0.3 ml. at Site of B Virus Inoculation 15 min. after 20 TCD_{50} B Virus Intracutaneously

	No. inoculated	No. died	Days to skin lesion	Days to paralysis	Days to death
Monkey G.G.	6	5	6 to 10	9 to 10	11 to 13
Human G.G.	5	5	5 to 6	8	10 to 12
Controls	4	4	5	8	9 to 11

Antibody levels and protection in animals. Four rabbits were given intramuscularly 5 ml. of the immune monkey gamma globulin. B virus antibody titer in the sera increased to 1:8 in 24 to 48 hours. Since a titer of about 1:5 might have been expected following intravenous injection of 5 ml., it would appear that all the gamma globulin was absorbed into the blood system.

To determine whether intramuscular injection of the immune monkey gamma globulin after infection could give any protection, groups of rabbits were inoculated (see TABLE 6). There appears to be a small measure of protection against minimal doses of virus after an interval of one hour, but intramuscular inoculation seems of relatively little value.

A series of experiments was carried out in an attempt to obtain protection by local injection of the immune serum. In the first experiment (TABLE 7) 0.1 ml. was injected intracutaneously in each of 5 sites surrounding the

virus inoculation after an interval of 15 min. No definite evidence of protection was obtained. Combined intracutaneous, subcutaneous, and intramuscular inoculation after an interval of 10 min. was then tried. The results are given in TABLE 8. The immune monkey gamma globulin gave protection against the small dose of virus, but human gamma globulin had no effect. The latter had a titer of 1:8 against B virus.

TABLE 8

PROTECTIVE EFFECT OF GAMMA GLOBULIN IN RABBITS

Intracutaneously, 0.5 ml.; 1.0 ml. Subcutaneously; 1.0 ml. Intramuscularly 10 Min. after 20 TCD_{50} B Virus Intracutaneously

	No. inoculated	No. died	Days to skin lesion	Days to paralysis	Days to death
Monkey G.G.	6*	0	—	—	—
Human G.G.	6*	6	5 to 12	9 to 16	13 to 17
Controls	2	2	6 to 7	8 to 11	10 to 13

* Three rabbits given 1 ml. gamma globulin intramuscularly 2, 4, and 6 days after challenge.

TABLE 9

PROTECTIVE EFFECT OF MONKEY GAMMA GLOBULIN IN RABBITS

Combination of Different Routes 10 Min. after 20 TCD_{50} B Virus Intracutaneously

Inoculation	No. inoculated	No. died	Days to skin lesion	Days to paralysis	Days to death
1.0 ml. I.M.	6	5	3 to 5	7 to 9	11
0.5 ml. I.C. 1.0 ml. S.C.	6	0	—	—	—
0.5 ml. I.C.* 1.0 ml. S.C. 1.0 ml. I.M.	2	2	13	15 to 27	18 to 29
Controls	2	2	4 to 5	9	11

* Thirteen to fourteen min. after B virus inoculation.

The subcutaneous inoculation appeared to be the important one. To confirm this inference the next experiment was done (TABLE 9). The group given the gamma globulin by all 3 routes, as a repetition of the previous experiment, is of particular interest. These animals, although expected to survive, succumbed after prolonged incubation periods. The most likely reason for the failure in these 2 animals was a slight delay in giving the gamma globulin. Under the conditions of this experiment, the interval of 10 min. may have been critical. In another experiment (TABLE 10) 3 of 4 rabbits given the gamma globulin subcutaneously survived.

Combined intracutaneous and subcutaneous administration gave complete protection. While this may have been due simply to the larger total dose, it may well have been due to better infiltration of all surrounding tissues

and lymphatic channels. The efficacy of the subcutaneous injection indicates that the important factor is the introduction of the immune serum into the lymphatic drainage before virus escapes. If so, blockage might be effective even farther along the lymphatic system.

These considerations lead to the suggestion that the application of a tourniquet might, by stopping lymphatic and venous return flow, prolong the effective interval between infection and the administration of immune serum. An experiment to test this hypothesis was done but failed to provide this information, as the rabbits given the monkey gamma globulin 20 min. after virus inoculation survived, as did those on which a tourniquet was also applied. This experiment is described because it shows the variability encountered in different experiments and indicates that under certain conditions the effective time interval may be at least 20 min. This measure is suggested as one which should be investigated.

Table 10
Protective Effect of Gamma Globulin in Rabbits
Monkey Gamma Globulin 1.0 ml. Subcutaneously 10 Min. after 20 TCD_{50} B Virus Subcutaneously

Rabbit no.	Days to skin lesion	Days to paralysis	Days to death
W-106	—	—	Survived
W-107	—	—	Survived
W-108	4	8	11
A-419	—	—	Survived
Controls			
W-109	5	10	11
W-110	—	10	12

Antibody titers in a human subject. Fifteen ml. of immune monkey gamma globulin was given intramuscularly to a human subject whose serum, tested repeatedly during the previous 2 years, at no time had shown any neutralization of small doses of B virus or herpes virus. A dose of 15 ml. was used, since the weight of the subject was 62 kg.; a dose of 20 ml. then might be expected to produce the same serum level in an individual weighing from 80 to 85 kg. The neutralizing titer of the serum at intervals of as much as 28 days is shown in FIGURE 2. The preinoculation sample, plotted as a titer of 1:1, was actually without any neutralizing effect. The titers against small virus doses, for which control tubes without serum were all positive, are shown because they give some additional estimate of the rate of rise and fall. The specificity is apparent from the data.

Since the titer of the monkey gamma globulin was 1:125 and the estimated ratio, vol. inoc.:vol. serum, was about 1:200, the activity could be expected to be detectable in undiluted serum only, if given intravenously. The observed titer of 1:2 is within the limits of this type of assay and, as in the rabbits, indicates that all the foreign gamma globulin was absorbed into the blood system.

The rate of decline appears to be similar to that of maternal antibody in

infants, indicative of a greater degree of compatibility between monkey and human serum than, for example, between horse and human serum. During the 2 months prior to the injection of 15 ml. of monkey gamma globulin, 5 small inoculations of 0.1 to 0.3 ml. had been given. The absence of reaction, however, was not of significance, as this subject had never shown sensitivity to any foreign protein. Two other persons have been given 15 or 20 ml. of the monkey gamma globulin without untoward effect. However, further work on the antigenic relationship between human and monkey sera is required before any estimation of the risk of sensitization on repeated administration may be made. For this reason we have not proposed routine use

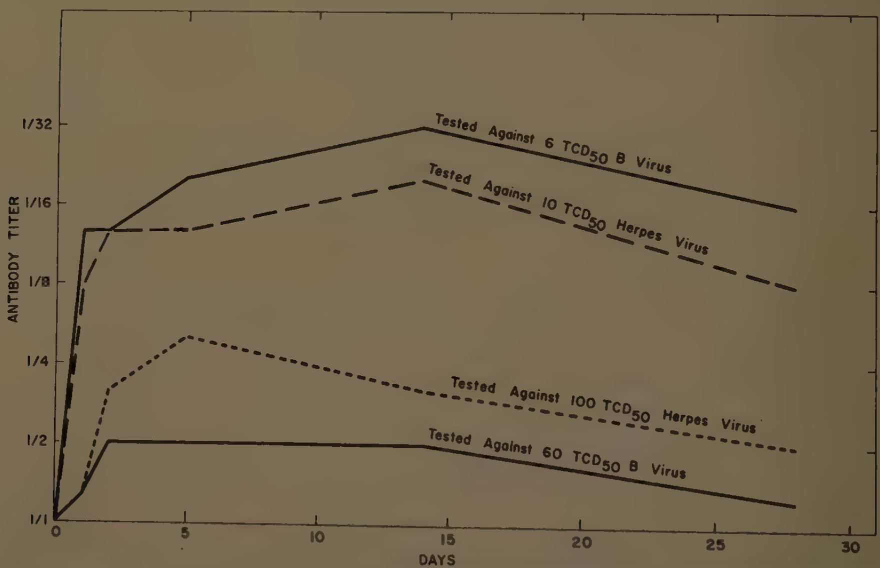

FIGURE 2. B virus antibody titer in serum of human subject after intramuscular inoculation of 15 ml. immune monkey gamma globulin.

of monkey gamma globulin but only when there has been exposure to a monkey with suspicious lesions, to one experimentally infected, or to B virus tissue culture fluids.

Summary

Experiments on active and passive immunization against B virus are described. The former have the objective of immunizing monkeys against the infection. The latter are directed toward the use of immune monkey gamma globulin for prophylaxis in humans after exposure.

Monkeys with antibody from previous infection, either natural or experimental, were resistant to reinfection by the cutaneous route. This observation confirmed earlier findings by others and gave a basis for attempting to protect against infection by active or passive immunization.

Formalin-inactivated vaccine was prepared. Multiple doses produced antibody and gave protection in rabbits. Evidence of antibody production but

no definite evidence of protection was obtained after three doses of vaccine in monkeys. The results suggested that a more potent and stable vaccine would give protection against infection by the cutaneous route.

A gamma globulin concentrate was prepared by ammonium sulfate fractionation of pools of immune monkey sera. In rabbits a relatively large intramuscular inoculation of the immune monkey gamma globulin gave some degree of protection when injected 1 hour after intracutaneous inoculation of minimal doses of B virus. Local injection within 10 min. gave protection against a small dose of virus. The degree of protection against larger doses is not known.

In 1 human subject without antibody against B virus or herpes simplex virus, a low level of antibody was produced by intramuscular inoculation of the immune monkey gamma globulin. Antibody could be detected 28 days after the injection.

The results in rabbits indicate that local infiltration of a wound with immune monkey gamma globulin within 10 min. would give some degree of protection in humans. Intramuscular inoculation might give some additional protection. Until more is known about the risk of sensitization, the use of monkey gamma globulin is proposed only when there has been exposure to a monkey with suspicious lesions, or to one experimentally infected, or to B virus tissue culture fluids.

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SUMMARY: CARE AND DISEASES OF THE RESEARCH MONKEY

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This monograph has covered a wide range of subjects, all quite pertinent to its title. It is apparent from the material presented that a wealth of knowledge has been acquired in the few years during which monkeys have become widely used in research. The contributors have covered such important points as the great number of monkeys used annually, their cost, their importance in research, the difficulty in defining a normal monkey, the problems associated with the rigors of transporting monkeys, and the high mortality rates frequently encountered.

In the first paper, F. A. Ulmer has given an excellent comparison of monkeys in captivity and those in their natural environment. His statement that "close confinement and resultant crowding have a drastic effect on all monkeys" is a significant one and should not be disregarded.

The paper by D. G. de Valois on the transportation of monkeys is thorough and deserves special attention. As long as it is necessary to transport monkeys in large numbers for great distances as economically as possible, certain problems will exist. However, until this first problem of making monkeys available for laboratory use is properly solved, untold hours of the time of skilled people and thousands of dollars will be spent in correcting damage done in transit. A number of the papers in this publication deal with the problems of correcting such damage. How much better it would be to spend the equivalent time, money, and effort in developing basic knowledge regarding the biology of the animals and in furthering research on healthy animals. The money expended in "conditioning" and "curing" imported animals could be spent better in preventing these needs through proper sorting, crating, and transporting.

Colony husbandry of large monkey colonies is vitally important. Basic principles of animal husbandry, including nutrition and sanitation, are of prime importance. However, they must be associated intimately with a thorough knowledge of the ecology of the animals themselves. It is apparent throughout these papers that too little knowledge of the ecology of monkeys has been at hand or, if available, the knowledge has been disregarded. We have only to reflect for a monemt on the years required to develop strains of white mice and rats that are sufficiently standardized biologically to meet the requirements of research workers. The same thing is true of domestic chickens. How long has it taken man to develop these birds to the point where they will live in close confinement in a totally unnatural environment and thrive on a completely man-made ration? To take monkeys from their wild habitat and acclimate them to laboratory colony conditions in a matter of hours is asking too much.

The papers dealing with skeletal growth, hematology, parasitology, antibacterial substances, electrocardiograms, neoplasms, oral diseases, diseases of

the nervous system, enteric bacteria, enteric viruses, pox diseases, and B virus infection all are important to our basic knowledge. The work done in these areas has been scholarly and revealing. The results of the investigations may be more significant than is now appreciated.

It is hoped that in the future an even greater advantage will be taken of the opportunity to study these and other phases of the ecology, anatomy, physiology, bacteriology, parasitology, and virology of the thousands of monkeys used each year. Research teams should be organized to make sure that full use is made of these animals.

The titles of the papers comprising this monograph show a noticeable lack of studies in histology. G. M. Krise's paper on hematology admirably points out the great variations obtainable in hematological research on primates.

Much work has been done on the significance of *Shigella* and *Salmonella* infections. There is no reason to suspect, nor evidence to prove, that these infections manifest themselves any differently in primates than in other vertebrates, and they are of no different significance. It long has been recognized that such infections are frequently associated with other contributing factors or agents.

The term stress and its significance should not be disregarded. The importance of stress is paramount in dealing with the diseases of such a highly evolved organism as a primate second in development only to *Homo sapiens*. The psychic and physical impact on monkeys of the type of handling they have received for purposes of research during the past two decades is bound to have stress implications of great importance.

This monograph clearly indicates the wealth of knowledge accumulated in a few short years. It also points out how much more can and must be learned through a continued and even more cooperative and concentrated endeavor.

Printed in the United States of America

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